Porcine reproductive and respiratory syndrome virus (PRRSV-1) recognition of peptide sequences in CD163 SRCR5

C. Williams, A.M.M. Stoian, R.R.R. Rowland

Department of Diagnostic Medicine and Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS

Introduction

- PRRSV is an important swine pathogen that uses macrophages as crucial target cells for viral replication.
- A macrophage specific molecule named CD163 was identified as a receptor for PRRSV.
- Cells that are non-permissive to PRRSV become permissive after transfection with CD163 constructs (Calvert et. al 2007).
- Using macrophages from CD163 SRCR5 knockout pigs Burkard et al. 2017, showed that SRCR5 is the receptor for both PRRSV-1 and PRRSV-2.
- Moreover, complete deletion of CD163 SRCR5 can produce pigs that are entirely resistant to PRRSV-1 infection (Rowland et. al 2012).
- However, CD163 has many important biological functions (Burkard et al. 2017).
- CD163 is responsible for homeostatic processes within the body such as uptake of excess hemoglobin in the blood and regulation of inflammation (Etzerodt et al. 2013).

Objective

- The purpose of this project is to the smallest mutation in SRCR5 that will prevent PRRSV-1 find infection but also conserve CD163's biological functions.
- The approach was to insert SacII sites along the SRCR5 polypeptide.

Materials & Methods

- Non-permissive HEK293T cells were transfected with CD163 cDNA constructs that contained SRCR5 mutations.
- The mutations were created by the insertion of a SacII site which codes for a proline arginine (PR) dipeptide.
- More specifically, each of these construct carries an insertion of Proline-Arginine (PR) dipeptides, coding for a SacII site, at every 30 bp along the SRCR5 cDNA (figure 1).
- Fusion of constructs to a green fluorescent protein (GFP) allowed for visualization of the proper expression.
- Each mutation was made in CD163 SRCR5 constructs to PRRSV-1 infection
- Cells expressing each mutant were infected with the Lelystad strain of PRRSV-1 which had a red florescent tag.
- Infection was visualized by IFA staining using a monoclonal antibody recognizing PRRSV-N protein.
- Results were recorded as percent infection of red IFA positive cells.

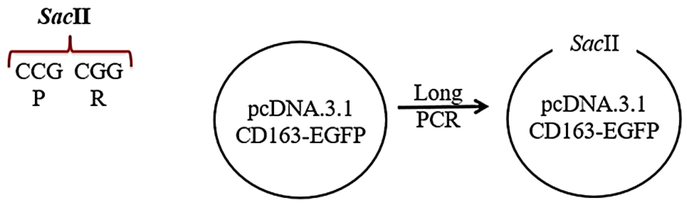


Figure 1. Visualization of the insertion of proline arginine (PR) dipeptides into the SRCR5 cDNA region of interest in CD163.

Results

	PRRSV-1
CD163	+++
PR-9	++
PR-15	+++
PR-22	++
PR-32	+
PR-38	+++
PR-42	++
PR-44	++
PR-48	++
PR-55	++
PR-58	+/-
PR-62	+++
PR-67	++
PR-78	+++
PR-89	+
PR-100	+/-

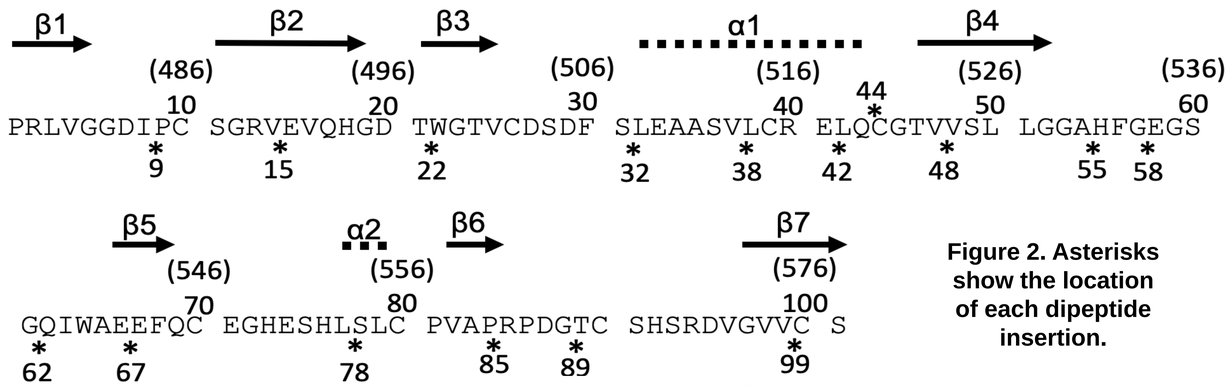
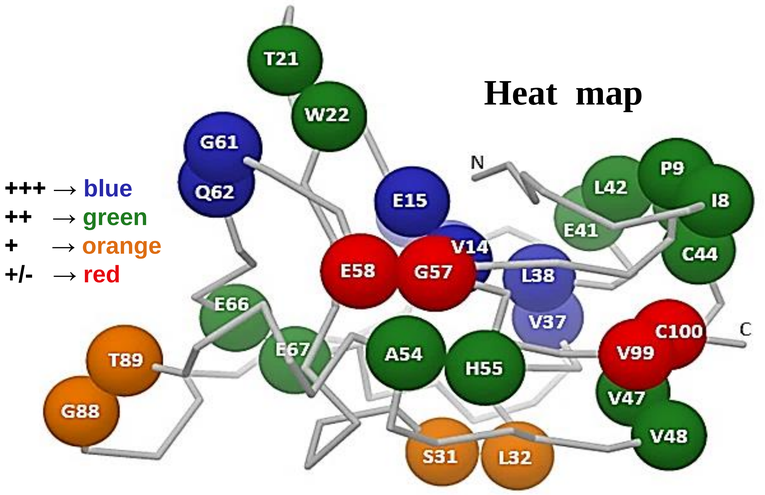


Figure 2. Asterisks show the location of each dipeptide insertion.

Figure 3. Predicted location of PR insertions in SRCR5. Each amino acid pair in the ribbon structure identifies the location of each PR insertion. The structures are based on the X-ray crystallography data deposited in RCSB Protein Data Bank (PDB code 5JFB) and viewed using UCSF Chimera (24).

+++ → blue
++ → green
+ → orange
+/- → red



Summary/Conclusion

- The infection trials revealed a wide range of infection rates, from the mutations that showed no or little effect to mutations that almost completely blocked infection.
- Proline arginine (PR) insertions in positions 15, 38, 62 and 78 had no effect on PRRSV-1 infection.
- PR insertions at positions 58 and 100 almost blocked infection of PRRSV-1.
- This data can be applied to the creation of a CD163 genetically modified pig that will retain a structurally intact and functioning CD163 SRCR5 that is resistant to PRRSV infection.
- These results show the possible contact regions between the PRRSV viral proteins and the CD163 receptor.

References

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Acknowledgments

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