Current Problems of PRRS and Alternative Approaches to Control

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Presentation Contents

1. The disease (PRRS) and the virus (PRRSV)

2. Current status of PRRS

3. Antiviral host response and type I interferons (IFNs-α/β)

4. Viral suppression of host antiviral response

5. Viral suppression of host gene expression

6. IFN-suppression-negative PRRSV as a future potential
Porcine reproductive and respiratory syndrome (PRRS)

First described in the US as ‘Mystery Swine Disease’ in 1987, and almost simultaneously but independently found in Europe.

Two distinct types;  Genotype I (European type)

Genotype II (North American type):

-1) Pregnant sows
  - Inappetence, lethargy, depression, high fever
  - Abortion, mummification, delivery of stillborns, weak-born
  - Some show respiratory distress
  - Delayed parturitions may occur

-2) Suckling pigs
  - Respiratory illness (thumping)
  - Growth retardation
  - Increased preweaning mortality of up to 40%

- 3) Boars- shedding in semen
Sow off-feed and born-dead pigs
Emergence of highly pathogenic PRRSV (HP-PRRSV) in China 2006

High fever of 41-42°C
High morbidity and high mortality of 20% up to 100%
Blue ear, abortion, severe respiratory symptoms, internal hemorrhages, and neurological signs
Spread to Vietnam, Thailand, Myanmar, and Russia

Pathological scores of the lungs of HP-PRRSV infected pigs

The severity of lung pathological lesions

The severity of lung fibrosis
Emergence of NADC30-like PRRSV CHsx1401 in China 2014: virulent but less pathogenic than HP-PRRSV

<table>
<thead>
<tr>
<th></th>
<th>NADC30</th>
<th>Henan-Xinx</th>
<th>MN184A</th>
<th>MN184B</th>
<th>VR2332</th>
<th>CH-1a</th>
<th>JXwn06</th>
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<td>CHsx1401</td>
<td>95.7%</td>
<td>93.0%</td>
<td>87.1%</td>
<td>87.4%</td>
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<td>84.8%</td>
<td>83.8%</td>
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Vaccination is a major control measure of PRRS

Commercial vaccines for HP-PRRSV MLV vaccines in China:

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<th>Parental</th>
<th>Attenuation</th>
<th>Protection</th>
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<td>MLV R98</td>
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<tr>
<td>MLV JXA1-R</td>
<td>JXA1</td>
<td>MARC-145 P82</td>
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<td>Yu, CVI 2015,22:493</td>
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<tr>
<td>MLV TJM-F92</td>
<td>TJ</td>
<td>MARC-145 P92</td>
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<td>MLV HuN4-F112</td>
<td>Hun4</td>
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<td>Yes</td>
<td>Tian, Vet Micro 2009,138:34</td>
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<tr>
<td>MLV GDr180</td>
<td>GD</td>
<td>MARC-145 P180</td>
<td>Yes</td>
<td>Liu, J Comp Path.153:38</td>
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</table>

Their safety? – mixed reports

JXA1: Guangdong Dahuanong Animal Health Products
R98 (classical type PRRSV strain), Jiangsu Nannong Hi-Tech Co
PRRS virus is a porcine arterivirus (ICTV 2017)

Order *Nidovirales (4 families)*

Family *Coronaviridae (2 subfamilies)*
- *Torovirinae* Berne virus, Breda virus
- *Coronavirinae* (Alpha-, Beta-, Gamma-, Delta-coronaviruses)

Family *Roniviridae* Okavirus

Family *Mesoniviridae* Insect viruses

Family *Arteriviridae (5 genera)*
- *Diparterirus* Wobbly Possum disease virus
- *Equarterivirus* Equine arteritis virus (EAV)
- *Nesarterivirus* African Oouched rat arterivirus
- *Simarterivirus* Simian hemorrhagic fever virus (SHFV) (10 species)
- *Porarterivirus* Lactate dehydrogenase-elevating virus (LDV) (4 species)
  - PRRS virus type 1
  - PRRS virus type 2
  - Rat aterivirus 1
Current problems in developing effective vaccines for PRRS

1. **Virus:**
   - Genetic and antigenic heterogeneity
   - Rapid evolution and quasi-species nature
   - Persistence
   - Potential reversion of vaccine virus to virulence

2. **Host:**
   - Poor induction of neutralizing antibodies
   - Role of cellular immunity for protection is unclear
   - Inadequate innate immune response
Trends of type 2 PRRSV diversity based on ORF5: Grouping of sequenced isolates (n>13,000) into 9 lineages

Leung et al. JGV, 2015; 96:1570
Genetic variability of isolates within major countries and regions impacted by type 2 PRRSV.
PRRSV genome organization and coding strategy

Single-stranded, positive-sense RNA of 15,411 nucleotides with a poly(A) tail of >72 A’s

Direct translation from the genome

Translation from sg mRNAs

Non-structural proteins

Viral membrane 4.5 nm
Core capsid 42 nm
Virion 52 nm

PP1a

PP1ab

Structural proteins

Cytoplasmic replication of PRRSV

Nucleus

Endosome

mRNAs

genome (+)

N

nsp1α

nsp1β
Induction of antiviral state in cells by type I IFNs

- PRRS virus-infected cell
- IFN production pathway
- Secretion of IFN-α/β
- Nucleus
- IFN signaling pathway
- Secretion of IFN-α/β
- IFN induces an antiviral state in uninfected cells
In virus-infected cell

IFN production pathway

PRRS virus-infected cell

Nucleus

RNA virus

Endosome

Mitochondria

RIG-I/MDA5

IPS-1

IRF3

CbP

Nucleus

IFN-β
In neighbor cells: JAK-STAT signaling pathway

IFN induces an antiviral state in uninfected cells

Secretion of IFN-α/β

Anti-viral immunity:
- ISG15, Mx, PKR, PML, OAS, >300 antiviral proteins
Pleiotropic effects of type I IFNs on immune cell responses


Type I IFNs

Epithelial cells
APC function
Antiviral defense
Reduced apoptosis

Monocyte
APC function, T cell differentiation, IFN/chemokine production

Macrophage

Dendritic cell

Type I IFNs

Apoptosis, proliferation

B cells
IgA, IgM, IgG

γδ T cells

Th2 cells

Th1 cells

T reg cells

CD8+ T cells

Cytotoxic T cell response
Memory response

NK cells

IL-17
In PRRSV-infected pigs, IFN response is inhibited

✓ Suppression of type I IFN-(α/β) responses:
  ▪ IFN concentrations are low in the lungs and sera of PRRSV-infected pigs (Albina et al., 1998)
  ▪ IFN-α levels are lower than expected in PRRSV-infected pigs (van Reeth and Nauwynck, 2000)
  ▪ IFN-α induction by PRRSV is poor in alveolar macrophages which are primary target cells for the virus (Lee et al., 2004)
  ▪ Microarray-expression of IFN-α/β genes is down-regulated in MARC-145 cells (Miller et al., 2004)
✓ Increased production of Interleukin (IL)-10
✓ Persistence of the virus in the tonsils for up to 6 months
✓ Involvements of type I IFN signaling and increased IL10 production in establishments of viral persistence (Nat Rev Immunology, 2013)
PRRSV suppresses IFN-α/β production

Real-time RT-PCR for IFNβ mRNA
PRRSV suppresses IFN-α/β production

IFN bioassays using vesicular stomatitis virus expressing GFP (VSV-GFP)

Sample dilutions

1/2  1/4  1/8  1/16  1/32

PRRSV(-)
poly(I:C)(-)

PRRSV(-)
poly(I:C)(+)

PRRSV(+)
poly(I:C)(+)

VSV did not grow
Presence of IFN

Green GFP(+)
VSV growth
Absence of IFN

Black GFP(-)
Identification of PRRSV proteins suppressing the IFN response
N, nsp1α, and nsp1β are nuclear-cytoplasmic proteins in PRRSV

5'cap

1a

1b

E

5a

3'UTR

A(n)

ZF1

PCPα

ZF2

PCPβ

nsp1α

nsp1β

N protein

GP4
PRRSV nsp1 orthologs in polyproteins of other (+)RNA viruses

**Pestivirus:**
BVDV (bovine viral diarrhea virus)  
CSF (classical swine fever virus; (2013, PLoS Pathog)

**Picornavirus:**
FMDV (foot and mouth disease virus) (2012, J. Virol)

**Alphavirus:**
Sindbis virus (2012, J. Virol)

**Coronavirus:**
SARS CoV, MERS CoV, Murine CoV (2007, PLoS Pathog)

**Arterivirus:**
PRRSV (2010, Virology)
IFN suppression-negative virus as a vaccine candidate

Picornavirus: FMDV (foot and mouth disease virus) (2012, J. Virol)


Equine arterivirus:

Swine influenza virus
Avian influenza virus

Mutation in Lpro
Deletion in nsp14
Deletion in nsp12
Deletion in NS2

NS2 gene
CBP (CREB-binding protein) is degraded by nsp1α protein

(J. Virol. 2009. 83:10931)
Zinc finger 1 motif in nsp1α mediates IFN suppression

Zn Finger 1

C C C C

C70 C76 H146 M

Zn Finger 2

1

PCPα

180

181

nsp1α

C8S

C8H

C10S

C10H

C25S

C28S

C8H/C10H

C8S/C25S

C8S/C28S

C10S/C25S

C10S/C28S

C70S

C70H

C76S

H146Y

M180I

M180H

C70S/C76S

C70S/H146Y

C70S/M180I

C76S/H146Y

C76S/M180I

Relative luciferase activity (Folds changes)

Virus Res. 2013. 172:54
CBP degradation and ZF1 mutants

- C8 and C25 in nsp1α are important for IFN suppression.
- But mutation of these residues make PRRSV non-replicating and non-viable.
Suppression of the JAK-STAT signaling by nsp1

IFN signaling pathway

IFN induces an antiviral state in uninfected cells

Secretion of IFN-α/β

Infected cell

Anti-viral immunity:
Summary I:

- **Nsp1α and nsp1β** function in the nucleus.
- Both proteins suppress host IFN responses.
- Functional sites in nsp1α have been determined.
- It is impossible to make replicating PRRSV.
Translational competition between host mRNA and plus-strand RNA virus genome
Non-viral (host cell) gene expression is inhibited by PRRSV

**Diagram Description:**
- **PRRSV**
- **Transfection**
- **Measurement of luciferase expression**

**Graphs:**
- Luciferase expression levels for Mock and PA8
- Firefly and Renilla expression levels for MOCK and PA8

**Transfection Components:**
- CMV
- IRES
- poly(A)
- Renilla
- Firefly
- TK
- Renilla
- β-actin
Accumulation of host mRNA in the nucleus in PRRSV-infected cells: hybridization for poly(A)+RNA using an oligo(dT) probe
Host mRNA nuclear retention is common for both genotypes.
nsp1β is the protein inhibiting mRNA export and protein synthesis

![Image of immunofluorescence staining for PIAS1, nsp1α, nsp1β, and N proteins with FLAG, Poly(A)+ RNA, and Merge columns.](image)

![Graph showing relative protein expression for GFP/β-actin with VSV-M, GST, nsp1α, nsp1β, and N treatments.](graph)
**Viral benefits**

1) Viral mRNAs in the cytoplasm are preferably translated to ensure efficient production of viral proteins and viral replication, whereas cellular mRNAs are retained in the nucleus.

1) Production of host antiviral proteins and cytokines is impaired, so that host responses may become inadequate and PRRSV replicates better in host.

Can we eliminate this function from PRRSV?
## Summary II

<table>
<thead>
<tr>
<th>Virus</th>
<th>Protein</th>
<th>Cellular distribution</th>
<th>Suppression</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Cytoplasm</td>
<td>Nucleus</td>
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<tr>
<td>PRRSV</td>
<td>nsp1α</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>nsp1β</td>
<td>++</td>
<td>+++</td>
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</table>
Identification of SAP domain in PRRSV nsp1β

**Nuclease catalytic site**
(Xue et al 2010. J.Virol)

**PLP1β**

**PRRSV nsp1β**

K18  E32  C90  SAP  H159

No effects on IFN antagonism

**126-LxxxLxxxGL-135**

Generation of SAP mutants

<table>
<thead>
<tr>
<th>Mutant</th>
<th>Sequence</th>
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<tr>
<td>PRRSV-VR2332</td>
<td>GYQTKHGVSGLKRQLQVNGLRAVTDLNG-PIVVQFFSVKESWI</td>
</tr>
<tr>
<td>PRRSV-PA8</td>
<td>GYQTKHGVSGLLRQVNGLRAVTDLNG-PIVVQFFSVKESWI</td>
</tr>
<tr>
<td>PRRSV-NVSL</td>
<td>GYQTKHVAGKSRLQVNGLRAVTDTDG-PIVVQYFVRESWI</td>
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<tr>
<td>PRRSV-P129</td>
<td>GYQTKHGVSGLLRQVNGLRAVTDLNG-PIVVQYFFVRESWI</td>
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<tr>
<td>PRRSV-BJ4</td>
<td>GYQTKHGVSGLLRQVNGLRAVTDLNG-PIVVQYFFVRESWI</td>
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<tr>
<td>PRRSV-JX143</td>
<td>GYQTKHGVPGLLRQVNGLRAVTDTHG-PIVIQYFSVENKESWI</td>
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<tr>
<td>PRRSV-HB-1/3.0</td>
<td>GYQTKHGVPGLLRQVNGLRAVTDTHG-PIVIQYFSVENKESWI</td>
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<tr>
<td>PRRSV-LV</td>
<td>GYRTPGVAGYGLQGRGLRRAVVPDPG-PIHEALSCQPSWI</td>
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<td>LDV</td>
<td>GYRTPGVAGYGLQGRGLRRAVVPDPG-PIHEALSCQPSWI</td>
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<td>SHFV</td>
<td>GYQLNCGVQGLIARRLGRLVQNGEKKFIAYTFHRGSWLI</td>
</tr>
<tr>
<td>EAV</td>
<td>RERQRTG----WGLSKTRLWLGLAG---LG-INASSGGLK</td>
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Aravind and Koonin (2000) TIBS
Wild-type nsp1β

SAP mutant L126A

SAP mutant L135A
Two SAP mutants are IFN-suppression negative

(A) IFN-β-Luc [Poly I:C]

(B) VSIV-GFP

Log$_2$ sample dilution folds /0.1mL
Generation of SAP mutant PRRSV and growth kinetics in MARC-145 cells

<table>
<thead>
<tr>
<th>Virus Titer (Log_{10} TCID_{50}/mL)</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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**WT-PRRSV**

**K124A**

**L126A**

**R128A**

**R129A**

**L130A**

**G134A**

**L135A**

**Graph:**

- **FL13**
- **K124A**
- **L126A**
- **R128A**
- **R129A**
- **L130A**
- **G134A**
- **L135A**

**Axes:**

- **Y-axis:** Virus titer (Log_{10} TCID_{50}/mL)
- **X-axis:** Time (0, 6, 12, 24, 48, 72, 96 h)
Infection study in pigs with SAP mutant virus

Intramuscular infection

1x10^4 TCID_{50} in 1 ml

2 ml per pig

4 groups, 10 pigs each group

Group 1: PBS
Group 2: SAP mutant 1 (vL126A)
Group 3: SAP mutant 2 (vL135A)
Group 4: WT PRRSV

Check PRRS-free

0  3d  7d  11d  15d  21d  27d

Examine:

Clinical signs (fever, body weights, coughing, behavior, necropsy)
Viremia
Virus neutralizing antibody
Serum antibody by ELISA
Virus persistence in tonsils
Serologic profiles of SAP-mutant PRRSV-infected pigs

Viremia

Neutralization Antibody

Days post infection

Days post infection
1. PRRSV inhibits type I IFN production and downregulates innate immunity, and subsequently modifies adaptive immunity in pigs. Nsp1α and nsp1β are two viral proteins antagonizing those functions.

2. Nsp1α degrades CREB-binding protein in the nucleus and inhibits IFN response during infection. Nsp1β blocks the nuclear export of host mRNA in the nucleus; 1) to utilize translational machinery for viral protein synthesis, and 2) to inhibit antiviral cytokine production.
3. The SAP motif in nsp1β is responsible for both IFN suppression and host mRNA nuclear retention. Mutations in the SAP motif confers the nsp1β-mediated IFN suppression-negative.

4. Two IFN suppression-negative PRRSV have been constructed. They result in the lower and shorter duration of viremia and higher neutralization antibodies in pigs.

5. Removal of IFN antagonism from PRRSV may be an alternative approach to new vaccines.
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