Foreign Animal Disease Research and African Swine Fever Vaccine Candidates

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Outline

• Foot and mouth disease (FMD)
  • Virus-host interactions – persistence – transmission – J. Arzt and C. Stenfedt
  • ‘FMD-LL3B3D vaccine development – E. Rieder  J. Hardham (zoetis)

• African swine fever (M. Borca and D. Gladue)
  • Vaccine candidates – research advances
  • Licensing and development of 3 current vaccine candidates
PERSISTENCE STUDIES

Clinical infection

FMDV clearance

≥28 dpi: NO persistence of infectious FMDV in porcine tissues
CLEARANCE OF PERSISTENT FMDV INFECTION REQUIRES ENHANCED PRO-APOPTOTIC AND CELLULAR IMMUNE RESPONSES

Carolina Stenfeldt, James Zhu, George Smoliga, Michael Eschbaumer, Luis Rodriguez, Jonathan Arzt
Transcriptome analyses results

**Acute phase (0-7 dpi)**
- Anti-Viral Pro-inflammatory Responses

**Transitional Terminators (10-21 dpi)**
- Activated antiviral response
- Activated cell-mediated immune response
- Promotion of Th1-associated responses
- Promotion of apoptosis

**Transitional Carriers**
- Inhibition of T cell activation
- Induction of cellular senescence
- Th2-associated cytokine production
- Inhibition of apoptosis
Can we create vaccines inducing desirable immune response to clear viral persistence?
FMDV transmission in Pigs

Informing FMD Modeling
Onset of infectiousness

Latent → Infectious

Detection of clinical FMD in donors

Timeline of Donor pigs

Infection → Transmission to contact exposed pigs

Timeline:

- Hours: 0, 8, 16, 24, 32, 40, 48, 56, 64
- Contact-exposed Pigs:
  - Hours 0: Not infected
  - Hours 8, 16: Not infected
  - Hours 24: Infectious
  - Hours 32, 40, 48, 56, 64: Infected

USDA
End of infectiousness

Window of transmission ca. 1-10 days post infection

Timeline of Donor pigs

0 Days
Infection
Transmission to contact exposed pigs
No transmission to contact-exposed pigs

Infectious
Not Infectious

Days
5
10
15

USDA

Infected
Not infected
Pig feed ingredients as potential vehicles for introduction of transboundary animal pathogens into the US

Each pigs (n=4 per group) received a measured dose of FMDV in 3 x 100g commercial pig feed
### FMDV challenge of pigs by feeding experimentally contaminated feed

<table>
<thead>
<tr>
<th>Dose ($\text{TCID}_{50}$)</th>
<th>FMDV A24</th>
<th>FMDV O/SKR/2010</th>
</tr>
</thead>
<tbody>
<tr>
<td>$10^8$</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>$10^7$</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>$10^6$</td>
<td>×</td>
<td>+</td>
</tr>
<tr>
<td>$10^5$</td>
<td>×</td>
<td>+</td>
</tr>
<tr>
<td>$10^4$</td>
<td>×</td>
<td>?</td>
</tr>
</tbody>
</table>
Novel Safe Platform For Production of FMD Inactivated Antigen Vaccines – CRADA Zoetis

Rapid vaccine production: easy swap of capsid sequences for new strains

SAFE
Attenuating factor (leader deletion) fully attenuated in cattle and swine

Dr. E. Rieder
21st Century vaccines

- Sequence acquisition
- Bioinformatic analysis, capsid design
- DNA synthesis – infectious clone platform
- Transfection of production cells
- Master stock production
- **Safe** Vaccine production

Done for FMDV3B3D, all serotypes, multiple subtypes

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19-20th Century vaccine production

- Virus acquisition
- Growth in susceptible cells
- Multiple passages in susceptible cells
- Adaptation of virulent virus to production cells – multiple passages in production cells (mutations?)
- Master stock production
- Vaccine production using virulent virus
Research Program

• Live attenuated vaccines
  • Existing vaccine candidates –
  • Continuing research – new vaccine candidates

• Subunit vaccines -

• Vaccine production cells

• DIVA test development

• Characterization of circulating strains
  • In endemic regions – Uganda
  • Epidemic regions - Vietnam
Timeline for Development of recombinant ASF viruses

- Production of the recombinant plasmid (RP) containing the reporter gene:
  - Left arm: p72 GUS
  - Right arm: 0kB X 0kB 180kB

- Successful recombination:
  - 2-3 months

- Plaque purification (6 to 30+ passages):
  - 3-10 months

- Produce stock and check absence of parental virus by PCR/NGS:
  - 2 months

- Check virus attenuation:
  - 3-4 months

- 2 years from concept to completion
CRISPR-Cas9 resulted in 4 log increase in initial recombination.

Fluorescent markers allow for faster purification.
Development of recombinant ASF viruses

Production of the recombinant plasmid (RP) containing the reporter gene

Successful recombination

Plaque purification

(30+ passages)

3+ passages

Produce stock and check absence of parental virus

Check virus attenuation

3-4 months from concept to virus
African Swine Fever Virus Georgia 2007 with a Deletion of Virulence-Associated Gene 9GL (B119L), when Administered at Low Doses, Leads to Virus Attenuation in Swine and Induces an Effective Protection against Homologous Challenge

- Protective at single, low dose
- Virulent at higher doses
- Further attenuation needed
- Licensed to 4 companies

ASFV-G Δ9GL (U.S. Patent No. 9,463,234)

African Swine Fever Virus Georgia Isolate Harboring Deletions of MGF360 and MGF505 Genes Is Attenuated in Swine and Confers Protection against Challenge with Virulent Parental Virus

- Protective at single low dose
- No clinical symptoms at higher doses
- MGF region prone to recombination
- Licensed to 4 companies

ASFV-G-ΔMGF (U.S. Patent No. 9,528,094)
Multiple-Gene Deletion Vaccine Candidates - ARS

ASFV-G Δ9GL-Δ9UK (U.S. Patent No. 9,808,520 and PCT/US2017/39277)
- Virus attenuation 100X over Δ9GL (10^7 HD_{50})
- Protective dose 10^4 HAD_{50}
- Onset of protection 14 days with 1 dose
- 100% protection against ASFV-G
- Licensed to 5 companies

ASFV-G Δ9GL-Δ9MGF360/505
- Too attenuated, does not replicate in Swine
- Results in inefficient antigen presentation
- Does not protect against challenge
Future actions

Include DIVA markers in our vaccine candidates

Continue evaluation of uncharacterized virus genes

ASFV Live Attenuated Vaccine Platform #3: ASFV-G-ΔXXX

- Novel genetic determinant of virulence: gen XXX
- Animals infected with $10^2$ HAD50 of ASFV-G-ΔXXX remained clinically normal during the 28 days observational period
- Animals infected with ASFV-G-ΔXXX remained clinically normal after the challenge with virulent ASFV Georgia strain

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of survivors/ total</th>
<th>Mean time to death (days ± SD)</th>
<th>No. of days to onset (days ± SD)</th>
<th>Duration (days ± SD)</th>
<th>Maximum daily temp ($^\circ$F ± SD)</th>
</tr>
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<tbody>
<tr>
<td>Mock</td>
<td>0/5</td>
<td>5 (0)</td>
<td>4 (0)</td>
<td>2 (0.0)</td>
<td>105.9 (0.55)</td>
</tr>
<tr>
<td>ASFV-Georgia-ΔXXX (3)</td>
<td>5/5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>102.42 (0.74)</td>
</tr>
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USDA- DHS ASF TASK FORCE
Research Working Group

• Problem
  • Live attenuated vaccines developed by ARS require a cell system for large-scale production
  • APHIS requires reliable source of cells for diagnostic virus isolation

• Solutions
  • Cell line exploration project (Gladue-ARS)
  • Primary peripheral blood derived macrophages (O’Donnell-APHIS)
  • Primary alveolar macrophages (Chung-DHS)
Working together

- Scientific staff
  - Jonathan Arzt, DVM, Ph.D.
  - Manuel Borca, DVM, Ph.D.
  - Douglas Gladue, Ph.D.
  - Teresa De Los Santos, Ph.D.
  - Fayna Diaz-SanSegundo
  - Elizabeth Rieder, Ph.D.
  - James Zhu, DVM, Ph.D.
- Support scientists -
- Admin staff -
- Research fellows / collaborators -
PIADC to NBAF Transition

Progress:
- Received budget ($5M) to start building up NBAF program
- 4 new SY positions to be filled in 2020
- 15 trainees – NBAF work force development
- Characterizing and selecting Biorepository for transfer (~1M tubes!!)
ARS VISIT TO MHK