Challenges for African Swine Fever Vaccine Development

D. L. Rock

dlrock@illinois.edu
An African Story...But
From Time-to-Time “Out of Africa”

Years of occurrence:
- Red: present in 2014 (as of Sept.)
- Yellow: present in 2011-13
- Green: present 4 to 15 years ago
- Dark green: present > 15 years ago

African swine fever status in Africa
An African Story...But
From Time-to-Time “Out of Africa”

- Sardinia, Italy
- Western Russia
- Caucasus Region
- Africa
- Netherlands, 1986
- Belgium, 1985
- France, 1964/68, 1974
- Malta, 1978-79
- Italy, 1967, 1983
- Brazil, 1978-81
- Portugal, Spain 1957, 1960-95
- Cuba, 1971, 1980
- Haiti, 1978-84
- Georgia, 2007
- China 20018
“African Swine Fever Sweeps Across Russia”
- And Pushes West

African swine fever confirmed in Belgium

This new outbreak may represent a new change in the epidemiologic situation of ASF worldwide, suggesting that the disease may have reached pandemic proportions.

Sep 13, 2018

African Swine Fever spreads to new region in Bulgaria

Virus found in wild boar 50km south of Romanian border

Recent African Swine Fever outbreaks domestic pigs and wild boar in 2017

[Insets: wild boar cases in Czech Republic and backyard outbreaks in Romania]
“African Swine Fever Sweeps Across Russia”
And Now China ..... 

Farming in China
Aporkalypse now

African swine fever hits the home of half the world’s pigs

China’s swine herd has halved to ~ 200 million head

The pigs now missing in China are greater than the rest of the world’s total production
ASF “Out of Africa” Forever

Endemic disease in over ½ of the world’s pigs
African Swine Fever Virus

- Sole member -Asfarviridae
- Icosahedral virus ~ 200 nm
- Linear dsDNA genome
  -185-193 kbp (157-168 genes)
- Replicates in cell cytoplasm
African Swine Fever Virus

Only DNA Arbovirus

Infects and persists in soft ticks and members of *Suidae* family
African Swine Fever

- Highly lethal (100%) to subclinical
- Edema, ascites and hemorrhage
- All domestic pigs susceptible
- Virulence-replication and spread within mononuclear-phagocytic system
## Forms of ASF - Clinical Signs and Symptoms

<table>
<thead>
<tr>
<th></th>
<th>Peracute</th>
<th>Acute</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virulence of strain</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td>Immune status</td>
<td>Death before seroconversion</td>
<td>Many die before seroconversion</td>
</tr>
<tr>
<td>Clinical signs</td>
<td>Often found moribund or dead</td>
<td>Febrile (40.5°C–41.5°C), leukopenia, anorexia, blood in feces, reluctant to move, erythemic skin progressing to cyanosis near death</td>
</tr>
<tr>
<td>Gross lesions</td>
<td>Death occurs before distinct lesions form</td>
<td>Spleen enlarged (up to 3 times normal), dark and friable; multiple hemorrhages of internal organs, especially kidneys and heart; hemorrhagic lymph nodes; edema of gall bladder and lungs; congestion of meninges and choroid plexus</td>
</tr>
</tbody>
</table>


**Acute Disease course 2 to 7 days with mortality rates approaching 100%**
Long-term latent infection with ASFV
Role in virus transmission?

- Established in ~ 100% of infected/surviving animals
- ASFV DNA detectable in monocytes/macrophages, bone marrow, tonsils, synovial fluid
- Infectious virus not detectable during latent phase
- Viral reactivation?
  - infrequent event in swine host?
  - biological significance in pig-tick cycle?
African Swine Fever Vaccine Design/Development

- No Vaccine Available
- Surviving pigs protected from homologous virus challenge
- Cross-protective immunity difficult to achieve
- No definitive immune correlates of protection
- Viral antigens responsible for protection undefined
- ASFV strain variation and variability unknown
- Vaccine for non-endemic regions must be DIVA compatible
ASF – Homologous/Heterologous Protective Immunity

- Heterologous virus cross-protection infrequent

- What is a heterologous virus? - boundaries of cross protection not clear
  - distant virus may protect
  - apparently closely-related virus may not
Live-Attenuated ASF Vaccines

ASF viruses that don’t cause disease but are capable of inducing protective immune response in the pig
Rational Engineering of Live Attenuated Virus Vaccines

~ 50% of viral genes associated with VHR
Most functions unknown

- **Tissue Tropism**
  - Attachment Proteins
  - Inhibitors of Apoptosis
  - Other Tissue-specific Factors

- **Immune Evasion**
  - Inhibitors of Humoral Responses
  - Interference with Interferon function
  - Inhibition of cytokines and chemokines
  - Modulation of MHC function
  - Modulation of CTL and NK function

VHR gene complement varies depending on virus
Development of Engineered ASF LAVs as Vaccines

ASFV Gene Function in Infection and Immunity
Development of Engineered ASF LAVs as Vaccines

ASFV Gene Function in Infection and Immunity

ASF gene function:
- Virulence
- Immunomodulatory
- Macrophage host range
- Apoptosis inhibitors
- Virion morphogenesis

Gene Function

Macrophage Host Range

Protection

<table>
<thead>
<tr>
<th>ASFV Gene</th>
<th>Gene Conservation</th>
<th>Affects Macrophage Growth in Vitro</th>
<th>Affects Pathogenesis/virulence</th>
<th>Affects Tick Host Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>TK</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>9-GL (Erv-1)</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>MGA (novel)</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
</tbody>
</table>

Safety and viral persistence questions remain

Challenges remaining:

- Identify attenuating mutations which function reliably in diverse ASFV genetic backgrounds
- Broaden cross protective potential
- Maximize safety without compromising protective immunogenicity
- Persistent infection?
**Subunit ASF Vaccines**

Noninfectious vaccine - contains protective viral antigens
Development of Subunit ASF Vaccines

Considerations:

- Complex virus – many viral proteins

- ASFV antigens responsible for protective immunity undefined - p30, p54, p72, p22, CD2v, and others implicated

  - issues: Vectors used, proper antigen conformation dose and route

- ASFV Strain Variation and Variability Unknown – needs to be addressed for a broadly cross-protective vaccines

Alejo et al. JVI, 2018
Does this method of analysis capture the actual biological diversity of ASFV?
ASFV HAI Serotypes

- ASFV hemadsorption inhibition (HAI) serogroups exist >8

- ASFV cross protective immunity may be sero-group specific

J Gen Virol 96: 866-73*
**ASFV CD2v and C-type lectin: A role in HAI serotype specificity and protection**

- **CD2v** (EP402R)  
  **C-type lectin** (EP153R)

  - Necessary/sufficient for ASFV hemadsorption (HA) in vitro

  - Among the most variable in the ASFV genome

  - Mediate HAI serologic specificity

ASFV CD2v and C-type lectin: A role in HAI serotype specificity and protection

- **CD2v** (EP402R)  
  **C-type lectin** (EP153R)

  - Necessary/sufficient for ASFV hemadsorption (HA) in vitro
  
  - Among the most variable in the ASFV genome
  
  - Mediate HAI serologic specificity

<table>
<thead>
<tr>
<th>ASFV Viruses</th>
<th>Anti-SG2 Reference Serum</th>
<th>Anti-SG4 Reference Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>SG2</td>
<td>1:64</td>
<td>&lt;1:2</td>
</tr>
<tr>
<td>SG4</td>
<td>&lt;1:2</td>
<td>1:32</td>
</tr>
<tr>
<td>SG2$^{SG4}$ CD2v/Lectin</td>
<td>&lt;1:2</td>
<td>1:32</td>
</tr>
<tr>
<td>SG4$^{SG2}$ CD2v/Lectin</td>
<td>1:64</td>
<td>&lt;1:2</td>
</tr>
<tr>
<td>Mock</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

# Identification of ASFV Protective Antigens Using InterSerotypic Recombinant (Chimeric) Viruses

<table>
<thead>
<tr>
<th>Immunization</th>
<th>Challenge Infection</th>
<th>Protection Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virus</td>
<td>Virus</td>
<td></td>
</tr>
<tr>
<td>SG2&lt;sub&gt;a&lt;/sub&gt;</td>
<td>SG2&lt;sub&gt;v&lt;/sub&gt;</td>
<td>++++</td>
</tr>
<tr>
<td>SG4&lt;sub&gt;a&lt;/sub&gt;</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>SG4&lt;sub&gt;a&lt;/sub&gt; + 1-SG2 protein</td>
<td></td>
<td>+/-</td>
</tr>
<tr>
<td>SG4&lt;sub&gt;a&lt;/sub&gt; + 2-SG2 proteins</td>
<td></td>
<td>+/++</td>
</tr>
<tr>
<td>SG4&lt;sub&gt;a&lt;/sub&gt; + 3-SG2 proteins</td>
<td></td>
<td>+++</td>
</tr>
<tr>
<td>SG4&lt;sub&gt;a&lt;/sub&gt; + 4-SG2 proteins</td>
<td></td>
<td>++++</td>
</tr>
</tbody>
</table>
Identification of ASFV Protective Antigens Using InterSerotypic Recombinant (Chimeric) Viruses

Landrace/large white  
30-35 kg

Immunize ($10^6$ - IM)  
21 days  
Boost ($10^6$ - IM)  
21 days  
Challenge ($10^3$ - IM)  
Monitor: 45-60 days

Two – four independent experiments  (N = 8-16 animals)
Survival Viremia
SG2a
SG2v
SG4a
SG4 CD2/Lectin
SG2 CD2/Lectin
Control

Immune responses to additional ASFV SAPs are necessary for full protection in pigs
Development of inactivated/subunit ASF vaccine

- ASFV SAPs, CD2v and C-type lectin – important for homologous protection
  - should be further evaluated and targeted for vaccine design/development.

- Immune responses to additional ASFV SAPs necessary to achieve protection in pigs
  - work needed to identify them.
ASF strain variation – What is a heterologous strain?

Is ASF protective immunity HAI serotype–specific?

- further work needed to determine robustness and genetic and antigenic bounds of cross protective immunity
Future Vaccine Prospects
Vaccines for Endemic and Non-Endemic Regions

Engineered ASF LAVs

- Use in endemic regions – permit disease control and reduced economic losses – reduce threat for disease-free regions

- Field trials with candidate LAV needed – ensure safety and evaluate efficacy under field conditions
Future Vaccine Prospects

Vaccines for Endemic and Non-Endemic Regions

Subunit/Vectored ASF Vaccines

- Possible with identification of key protective antigens
  - likely serotype-associated antigens

- Suitable for use in non-endemic regions under emergency conditions – noninfectious/DIVA compatible
“Out of Africa” - which ASF virus is next?

ASFV Diversity - 22 genotypes/ 8-11+ serogroups defined
Shuhong Lu
Sushil Khatiwada
Sabal Chaulagain
P. Nagendraprabhu

Claudio Afonso
John Neilan
M. Borca
Z. Lu
Gerald Kutish
Edan Tulman
Laszlo Zsak

Sushil Khatiwada
Gustavo Delhon
Gerald Kutish
Edan Tulman
Galina Burmakina
Denis Kolbasov
Alexander Malogolovkin
Nikolai Salnikov

Federal Research Center of Virology and Microbiology, Russian Federation

University of Illinois

University of Connecticut

University of Nebraska - Lincoln

pork checkoff

United States Department of Agriculture
National Institute of Food and Agriculture