Pathogenesis of Pulmonary Disease In Ebola Virus-Infected Pigs

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Introduction

Family Filoviridae

Species: Ebola virus (EBOV) & Marburg virus

Enveloped, negative-strand RNA viruses

Pleomorphic, filamentous, 80nm diameter, varying lengths up to 14,000 nm

EBOV causes ebolavirus hemorrhagic fever (EHF)

EHF probably the most severe of viral hemorrhagic fevers
History And Distribution Of Reported EBOV Outbreaks

ZEBOV = Zaire ebola virus (1976)
SEBOV = Sudan ebola virus (1976)
REBOV = Reston ebola virus (1989)
CIEBOV = Cote d’Ivoire ebola virus (1994)
BEBOV = Bundibugyo ebola virus (2007)

> What possessed them to perform this seemingly *odd experiment*? *Bausch D.* *The Journal of Infectious Diseases* (2011)
Ebola In Pigs: Reston Ebola Virus

- Multiple outbreaks of respiratory and abortion syndrome in pigs in 2008
- Detection of porcine reproductive & respiratory syndrome virus (PRRSV)
- CPE in Vero cells suggested presence of another virus other than PRRSV
- The unknown virus identified as REBOV by EM & molecular biology techniques
- REBOV only detected in pigs co-infected with PRRSV

Reston Ebola Virus (REBOV) In Pigs In The Absence Of Co-infection

- Virus isolated from lungs, lymph nodes, nasal turbinates and muscle

- Gross & histopathology in the respiratory system and lymphoid tissues

- No clinical signs of disease

Experimental Zaire Ebola Virus (ZEBOV) Infection In Pigs

- Pigs infected with $10^6$ PFUs ZEBOV by oro-nasal routes
- Fever starting at dpi 4
- ZEBOV replicated mainly in the lungs
- Oral and nasal shedding of virus
- Severe pathology in the lungs
- Symptoms mainly respiratory
- Infected pigs transmit ZEBOV to naïve pigs

Objective

Main clinical sign in ZEBOV infected pigs: respiratory distress (due to pathology in the lungs)

Identify the mechanisms involved the development of pulmonary disease in ZEBOV-infected pigs
Pulmonary Innate Immune Responses In ZEBOV-infected Pigs

(Kobinger et al, 2011)
Pulmonary Cytokine Response In ZEBOV Infected Pigs

<table>
<thead>
<tr>
<th>Gene</th>
<th>Description</th>
<th>Fold change</th>
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<td>SPP1</td>
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<tr>
<td>SELL</td>
<td>Selectin</td>
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<tr>
<td>RETN</td>
<td>Resistin</td>
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<tr>
<td>PLUNC</td>
<td>palate, lung and nasal epithelium associated</td>
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**Attract monocytes, neutrophils & lymphocytes to sites of infection**

**Induce the secretion of chemokines & acute phase proteins**

**Drive differentiation and proliferation of immune cells**

**Cell adhesion & trafficking**

**Antinflammatory (IL-10)**
### Pulmonary Chemokine Response In ZEBOV-infected Pigs

<table>
<thead>
<tr>
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<td>AMCF-II</td>
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<td>Serum amyloid A2</td>
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<td>C9</td>
<td>Complement component 9</td>
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Attract monocytes, neutrophils & lymphocytes to sites of infection
## Upregulation Of Proapoptotic Genes In Lungs Of ZEBOV-infected Pigs

<table>
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<td>Allograft inflammatory factor</td>
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*Induce apoptosis of virus infected cells*

*Caspases also induce inflammation*
### Receptors And Interferon Stimulated Genes In Lungs From ZEBOV-infected Pigs

<table>
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<tr>
<th>Gene</th>
<th>Description</th>
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<td>Toll-like receptor 2</td>
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<td>TLR4</td>
<td>Toll-like receptor 4</td>
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<td>TLR6</td>
<td>Toll-like receptor 6</td>
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<tr>
<td>RIG-I</td>
<td>Retinoic acid inducible protein I</td>
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<tr>
<td>IRF1</td>
<td>interferon regulatory factor 1</td>
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<td>IRF7</td>
<td>interferon regulatory factor 7</td>
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<td>ISG15</td>
<td>Interferon-induced 15 kDa protein</td>
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<td>ISG20</td>
<td>Interferon-stimulated gene 20 kDa protein</td>
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<td>IFIH1</td>
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<td>IFIT-1</td>
<td>Interferon induced with tetratricopeptide</td>
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<td>MX1</td>
<td>Myxovirus (influenza virus) resistance 1</td>
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<tr>
<td>OAS2</td>
<td>2’5’-oligoadenylate synthetase 2</td>
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<td>IRG6</td>
<td>Inflammatory response protein 6</td>
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**PRR → innate immunity**

**ISG → antiviral response**
Model For The Pathogenesis Of Pulmonary Disease In ZEBOV-infected Pigs

ZEBOV in lungs:
Target cells: alveolar macrophages, DCs, epithelial cells

Cytokine response:
Proinflammatory (IL-1, IL-6, IL-8, IL-12, IL-22, IL-26, IL-27, GM-CSF, TNF-α, IFN-γ, selectin, resistin, SPP1)
Antiinflammatory (IL-10)
Suppressed (IFN-α)

Chemoattractants:
Chemokines: (CCL2, CCL3L1, CCL4, CCL8, CCL10, CCL19, CCL20 and AMCF-II);
Acute phase proteins (SAA, C9)

Interferon stimulated genes and transcription factors
(ISG15, ISG20, IFIH1, IRF7, vLIG1, IFIT1, MX1, MX2, OASL, OAS2, IRG6, DDX58)

Immune cell infiltration into lungs: Macrophages, neutrophils, lymphocytes

Apoptosis/necrosis: CTL, GZMB, CASP1, C9, TNF

Inflammation & pathology → respiratory distress

Anti inflammatory and modulation of T cell activation (IL-10, LAG-3)

Antiviral state and possible modulation of lung pathology
Aberrant host immune response induced by highly virulent PRRSV identified by digital gene expression tag profiling. Xiao et al. BMC Genomics (2010)
Are The Effects Of ZEBOV infection In Pigs Restricted To The lungs?

ZEBOV detection in tissues from infected pigs

<table>
<thead>
<tr>
<th></th>
<th>SLN</th>
<th>BLN</th>
<th>MLN</th>
<th>Lung</th>
<th>Liver</th>
<th>Spleen</th>
<th>Trachea</th>
<th>Muscle</th>
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<tr>
<td>Pig 7</td>
<td>4.95</td>
<td>5.99</td>
<td>0</td>
<td>3.73</td>
<td>4.17</td>
<td>0</td>
<td>4.39</td>
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<td>Pig 8</td>
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<td>9.52</td>
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<td>Pig 9</td>
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<td>0</td>
<td>9.54</td>
<td>4.94</td>
<td>4.39</td>
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<td>Pig 10</td>
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<td>Pig 12</td>
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<td>5.36</td>
<td>5.53</td>
<td>5.57</td>
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</table>
Changes In PBMC Subsets In ZEBOV-infected Pigs

- **MO**
  - % CD172a+ cells vs Days post infection

- **DC**
  - % dendritic cells vs Days post infection

- **B-cells**
  - % CD21+ cells vs Days post infection

- **T-cells**
  - % CD3+ cells vs Days post infection

Monocytes infected in vitro

B & T cells not infected
Systemic Cytokine Response In ZEBOV-infected Pigs

IFN-α

Days post-infection

Days post-infection

IL-6

pg/mL

pg/mL

IL-12

Days post-infection

Fold change in mRNA in PBMC

Days post-infection

Fold change in mRNA in PBMC

TNF-α

IFN-γ

Days post-infection

Fold change in mRNA in PBMC

Days post-infection

Fold change in mRNA in PBMC
IgM Antibody Response In ZEBOV-infected Pigs
**Summary**

**Systemic effect of ZEBOV**
- Fever
- PBMCs: ↓MO/DC, B cells
- Cytokines: IL-6, IFN-α, IFN-γ, IL-12, TNF-α
- Antibody: IgM, neutralizing antibodies

**Pulmonary effect of ZEBOV**
- Ebola virus
- BRONCHUS
- ALVEOLUS
- Epithelial cell
- Neutrophil
- Monocyte
- Cytokines, other mediators
- Blood capillary
“one should keep an open mind for the existence of other reservoir species and a role for potential amplifying hosts, especially after the discovery of Reston Ebola virus in pigs in the Philippines”: Feldman H and Geisbert T, 2011
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