The Lung Endothelium as a Target for Therapy during Severe Influenza

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Swine Flu Cases Worldwide Exceed 2,300

Mexicans, quarantined in China with no flu symptoms, landed Wednesday in Mexico City.

By DONALD G. McNEIL Jr.
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The World Health Organization said Thursday that 2,371 people in 24 countries now had confirmed cases of swine flu.
Influenza – one mutant strain away from disaster?

- The development of a highly transmissible and virulent strain can occur
- Resistance to existing antivirals may develop rapidly
- New therapeutic approaches are needed
Patient who die from influenza fall into 2 groups

- Primary viral pneumonia
- Secondary bacterial pneumonia
  - *S. aureus*, *S. pneumoniae*
Patient who die from influenza fall into 2 groups

- Primary viral pneumonia
- Secondary bacterial pneumonia
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The final common pathway?

**Acute lung injury**
Alveolocapillary membrane

Alveolar epithelium

Lung Endothelium

lumen
Influenza replicates in lung endothelium
IMMUNOSTAIN FOR VIRAL NUCLEOPROTEIN
Measurement of Leak

- Transendothelial Electrical Resistance (TEER)

Add fluorescein-tagged dextran to the upper chamber and allow 40 min for dextran flux. The measured resistance is $R = 24 \ \Omega \ \text{cm}^2$. 

Top electrode

Bottom electrode

R = 24 Ω cm²
Measurement of Leak

- **Dextran flux**

  1. Add fluorescein-tagged dextran to the upper chamber.
  2. Allow 40 min for dextran flux.
  3. Measure Fluorescence.
Influenza induces endothelial permeability

H3N2 (X31)
Influenza induces endothelial permeability

**Clinical Isolate (H3N2)**

H3N2 (X31)
Influenza induces endothelial permeability
What is the mechanism?
Influenza induces apoptosis
Blocking apoptosis partially attenuates flu-mediated leak
Signaling?
Replication-deficient influenza can still infect lung endothelium.

- **No flu**
- **Live flu**
- **UV flu**

**TCID50/mL**

**CONTROL**

**UV FLU**

Viral protein
Replication-deficient influenza induces endothelial permeability
Replication-deficient influenza does not induce apoptosis
Replication-deficient influenza causes a loss of claudin-5
No effect on VE-cadherin
Flu causes dose-dependent loss of claudin-5
Decreased claudin-5 may be due to...

- Decreased production
- qPCR shows no difference in mRNA levels

<table>
<thead>
<tr>
<th>UV flu</th>
<th>Control</th>
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<tbody>
<tr>
<td>$2^{-22.6-18.4}$ = 0.053</td>
<td>$2^{-22.8-18.2}$ = 0.040</td>
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<tr>
<td>$2^{-24.7-20.1}$ = 0.042</td>
<td>$2^{-23.9-19.1}$ = 0.038</td>
</tr>
<tr>
<td>$2^{-23.9-19.5}$ = 0.048</td>
<td>$2^{-23.8-19.5}$ = 0.051</td>
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Mean ± SE = 0.047 ± 0.003
Mean ± SE = 0.043 ± 0.004
Decreased claudin-5 may be due to...

- Increased degradation
Claudin-5 is not degraded in lysosomes

20-hour UV flu treatment

Claudin-5

LAMP-1

Merged
Protein to be degraded → Ubiquitinated protein → Proteasome → Protein entering a proteasome → Proteasome and ubiquitin to be recycled → Protein fragments (peptides)

http://bio1151.nicerweb.com/Locked/media/ch18/proteasome.html
Claudin-5 is not polyubiquitinated
Is Claudin-5 degraded by matrix metalloproteases?

http://www.uniovi.es/mrq/Ingles/mrq_MMPs.htm
Inhibition of matrix metalloproteases prevents the loss of claudin-5
Is the loss of claudin-5 required for flu-induced leak?
Over-expression of claudin-5 is sufficient to block flu-induced leak
cAMP stimulates claudin-5 synthesis
cAMP prevents claudin-5 loss
Formoterol can stimulate claudin-5 synthesis
Formoterol can induce claudin-5 expression
Formoterol can block flu-induced claudin-5 loss
Formoterol blocks flu-induced leak
Flu induces lung injury in mice
...that is attenuated by formoterol
Summary

- Human influenza can infect and replicate in lung microvascular endothelium
- Infection increases lung endothelial permeability
  - Endothelial apoptosis
  - Loss of claudin-5 (apoptosis-independent)
- Enhancement of endothelial barrier integrity may represent a novel therapeutic approach for severe influenza
- *Plos One* 2012 7(10):e47323
Ongoing Work

- Patient who die from influenza fall into 2 groups
  - Primary viral pneumonia (20%)
  - Secondary bacterial pneumonia (75%)
    - *S. aureus, S. pneumoniae*
Superinfection after influenza

• Autopsy data from 1918 found bacterial overgrowth
  • An era of no antibiotics

• An animal study of *S. aureus* superinfection found that bacterial overgrowth did not explain mortality (Iverson et al., *JID* 2011)
Does influenza predispose to acute lung injury from *Staphylococcus aureus*?

Stay tuned...
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