Treating the Host Response to Severe Influenza: Will it Work?

David S. Fedson
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Instead of Targeting the Virus with Vaccines and Antivirals, Could We Manage a Pandemic by **Treating the Host Response**?

**Influenza Virologists and Three Critical but Unanswered Questions**

1. **What characteristic is shared by most patients** of any age who are at risk of influenza-related mortality?
2. **What mechanisms** are responsible for influenza-related acute lung injury, ARDS, multi-organ failure and death?
3. **What explains the different mortality rates of children** and young adults in the 1918 and more recent influenza pandemics?
### A Common Feature Underlying All High-risk Conditions for Severe Influenza

<table>
<thead>
<tr>
<th>Low-grade inflammation</th>
<th>Low-grade immunosuppression</th>
</tr>
</thead>
</table>

- **Older adults**
  - Cardiovascular diseases: ++ - -
  - Chronic pulmonary diseases: ++ - -
  - Diabetes mellitus: ++ - -
  - Chronic renal diseases: ++ - -

- **Younger adults**
  - Obesity: ++ - -
  - Asthma: - - ++
  - Pregnancy: - - ++

Most of these conditions show evidence of **metabolic syndrome**
All of them share one common feature
an altered “innate immune rheostat”
Influenza Virologists and Their Understanding of the Host Response to Infection

- Influenza virologists focus on the initiation of infection (receptor binding, transmissibility), followed by a “cytokine storm” that is counteracted by CD8+ (and CD4+) T cells.
- Antibody responses eventually control virus replication → survival.
- Virulence factors intrinsic to the virus result in failure to control virus replication → complications and death.

Successful control of influenza requires targeting the virus with vaccines and antiviral agents.

The Host Response as Understood by Sepsis Scientists

- Early hyperinflammatory response - Th-1, Th-17; IFNγ, IL-1, IL-6, TNFα
- Late immunosuppression - Th-2, Treg; IL-10, TGF-β, lymphocyte apoptosis, secondary bacterial infection (<50%), multi-organ failure and death
- Resolution and recovery - active process, e.g., lipoxin-A4
- In fatal cases of sepsis and influenza, the median duration of illness is 10 days
- Most patients do not die during the early “cytokine storm” but later when they are immunosuppressed

Inability to restore homeostasis → multi-organ failure & death

Pandemic Influenza in 1918
Young Adults and Children
The “W-shaped” Mortality Curve of the 1918-1919 Influenza Pandemic

- Older adults protected by long-lasting immunity acquired by infection with an H1N1-like virus before 1889 (> 30 yrs of age in 1918)
- Mortality especially severe among young adults

- “relative resistance … (of children) … is associated with an enhanced innate immune response …(and) … for reasons that are as mysterious today as they were in 1918, (they) were able to cope with the disease much better than (adults).”

What is “enhanced” or “mysterious” about the innate immune response of children?

Clinical Influenza and Pneumonia Mortality in Children and Young Adults in the 1918 Influenza Pandemic

Infectious Disease Mortality Is Lower in Children than in Adults

Adapted from Kobzik L, unpublished compilation
IL-1β-stimulated Peritoneal Macrophages in Adults and Children

An anti-inflammatory (IL-10) response is dominant in children

Milestones in the Lifespan of Individuals
Consequences for the Species

- Individual incapable of reproduction; survival of no consequence to the species
- Individual capable of reproduction; survival of great consequence to the species

Birth ↓ Puberty/Menarche ↓ Death

↑ Growth is imperative; vigorous host defense is unnecessary

↑ Growth is completed; vigorous host defense to ensure reproduction is essential
Influenza Virologists and the Three Critical Questions: Insights from Other Scientists

- What characteristic is shared by most patients of any age who are at risk of influenza-related mortality?
  
  Patients at risk have an altered “innate immune rheostat” that is set at a different and more precarious level.

- What mechanisms are responsible for influenza-related acute lung injury, ARDS, multi-organ failure and death?
  
  Influenza is a multi-system disease that eventually involves cytokine dysregulation, impaired resolution of inflammation, endothelial dysfunction, alterations in energy metabolism and failure to restore mitochondrial biogenesis.

- What explains the different mortality rates of children and young adults in the 1918 and more recent influenza pandemics?
  
  Young adults have an aggressive host response to influenza that is sometimes self-destructive, whereas children have a more benign response that is less self-destructive and allows them to live.
Virus Levels or the Host Response? 
NK Cells, IL-15 and the Immunopathology of Influenza

- IL-15 influences the response of CD8+ and NK cells to influenza
- C57Bl/6 mice infected with PR8
- IL-15 -/- and NK cell-depleted mice → ↓ IL-6 and ↑ IL-10
  ↓ acute lung injury
  ↑ survival
  No difference in lung virus levels compared with controls

One of many examples
Modifying the host response improves survival with no change in virus levels

“In the complex interplay between host and influenza virus, host factors play an important part in disease severity and outcome, and studies focusing on identifying the human susceptibility factors are key for understanding host determinants of influenza virus pathogenesis.”


Well yes, of course!!!

More than 95% of influenza patients recover on their own, and only small numbers develop severe disease and die

Host factors are not just key factors; they are overwhelmingly the most important factors that determine outcome.

Could we treat the host response to influenza and save lives?
Treating the Host Response Is Complicated

“To realize the potential … (of) … targeting innate immunity, … (we must) … understand this process in considerably more detail. Research areas that warrant more attention are …

- systems biology and translational approaches … (that include) … TLR biology, deciphering the impact of modulators regulating inflammation, and of regulatory molecules balancing inflammatory responses, and deciphering the complex mechanisms of key human genetic mutations that alter disease susceptibility.

This information is imperative (sic) to enable the orderly and safe development of effective therapies …”

Targeting the Host Response: The TLR4-MAPK-NF-κB Network

• What is the target of immunomodulatory treatment?
• Is it imperative that we explain the complex molecular biology of the host response before learning how to manage influenza and sepsis?
• Could recognizing phenotypic changes with treatment give us a new approach to managing severe influenza?


Edward Jenner knew nothing about the germ theory, but by recognizing a different phenotype in milkmaids, he understood that vaccination could be used to manage smallpox.
Early Statins Reduce Mortality in Patients With ST-elevation Acute Coronary Syndrome

- Observational study of 10,484 patients with acute coronary syndrome (ACS)
- Among 8197 first-day survivors, 1426 (17%) received statins
- Statins significantly reduced all-cause 30-day mortality (propensity adjusted HR = 0.34) and ST-elevation ACS mortality (HR = 0.17)
- Benefits due to plaque stabilization, ↑ endothelial function, ↓ monocyte recruitment and ↓ prothrombotic state

Treating the Host Response to Severe Influenza: Will it Work?

Pandemic Influenza: A Potential Role for Statins in Treatment and Prophylaxis

David S. Fedson

The next influenza pandemic may be imminent. Because antiviral agents and vaccines will be unavailable to people in most countries, we need to determine whether other agents could offer clinical benefits. Influenza is associated with an increase in acute cardiovascular diseases, and influenza viruses induce proinflammatory cytokines. Statins are cardioprotective and have anti-inflammatory and immunomodulatory effects, and they thus might benefit patients with influenza. This hypothesis should be evaluated by using administrative databases to search for reduced rates of hospitalization and death due to influenza-related conditions among people taking statins. These studies should be followed by laboratory studies of statins in animal and cell-based models of influenza virus infection and, later, by clinical trials. Positive results from such studies would provide physicians in all countries with something to offer patients for treatment and prophylaxis of pandemic influenza. Generic statins will be widely distributed and inexpensive. They might be the only agents that could alter the course of a global pandemic.

Statin Treatment of Patients Hospitalized with Laboratory-confirmed Seasonal Influenza

• Retrospective cohort study of laboratory-confirmed influenza in 3043 hospitalized adults in 10 states in 2007-2008
• 1013 (33.2%) patients received statins, 881 (87% in hospital)
• 151 (5.0%) died in hospital or within 30 days of discharge
• Multivariate logistic regression model adjusted for age, race, cardiovascular, lung and renal disease, influenza vaccination and antiviral treatment

<table>
<thead>
<tr>
<th>Increased risk of death</th>
<th>(95% CI)</th>
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<tr>
<td>Inpatient statins</td>
<td>0.59</td>
</tr>
</tbody>
</table>

Statin treatment (87% inpatient) reduced mortality in the hospital and within 30 days of discharge by 41%

A “Family” of Immunomodulatory Agents That Might Bring About Phenotypic Improvement in Severe Influenza

- **HMG-CoA inhibitors** (statins)
  - ↓LDL cholesterol and prevent cardiovascular and cerebrovascular diseases
- **PPAR\(\alpha\)^* agonists** (fibrates)
  - regulate lipid metabolism, fatty acid oxidation
- **PPAR\(\gamma\) agonists** (glitazones)
  - increase sensitivity to insulin
- **AMPK agonists** (e.g., metformin, also glitazones)
  - act on PGC-1\(\alpha\) and improve energy metabolism
  
^*Peroxisome proliferator-activated receptor

There is substantial molecular “crosstalk” among these agents anti-inflammatory and immunomodulatory agents

Statins and PPAR and AMPK Agonists Possible Cell Signaling Effects in Treating Severe Influenza

- ↓ TLR4, NF-kB and AP-1; ↑ p38 MAPK and PI3/Akt (cytoprotective)
- ↓ pro-inflammatory TNFα, IL-1b, IL-6, IL-8, MCP1, MIP1α/β, CCR5
- ↑ anti-inflammatory IL-10, TGF-β
- ↑ pro-resolution lipoxin A4, resolvin E1
- ↑ HO-1 → ↓ TLR signaling for PAMPs, alarmins (DAMPS), ↓ cytokines
- ↓ cellular adhesion molecules (VCAM-1, ICAM-1, P-selectin)
- ↓ tissue factor, ↑ thrombomodulin → ↓ pro-thrombotic state
- ↓ iNOS, ↑ eNOS → stabilize iNOS/eNOS ratio
- modify macrophage function, caspase activation (inflammasomes) and autophagy and apoptosis in different cells
- ↑ GSH, ↓ NADPH oxidase (Nox), ROS and ↓ oxidative stress
- stabilize endothelial cell actin cytoskeleton and EC adherens junctions → ↑ pulmonary barrier integrity, ↓ endothelial leak
- ↑ PGC-1α → ↑ mitochondrial biogenesis, ↑ ATP
Four Examples of **Mechanisms Involved in the Pathogenesis of Severe Influenza and the Response to Immunomodulatory Agents**

- TLR4 signaling activates NF-kB → ↑ **pro-inflammatory cytokines** TNFα, IL-1β, IL-6, IL-8, MCP1, MIP1α/β, CCR5

- Resolution of inflammation is an active process, involving **lipoxin A4**, resolvin E1 and other pro-resolution factors

- Pulmonary **endothelial cells** determine microvascular **barrier integrity**, and barrier breakdown leads to ARDS, multi-organ failure and death

- Survival in critical illness is determined by early restoration of **mitochondrial biogenesis**
Common Cell Signaling Pathways in Mice With Acute Lung Injury (ALI)

- In acid and LPS-induced ALI, cell signaling proceeds from TLR 4 to NFκB → ↑ inflammatory cytokines
- Local generation of ROS → oxidized phospholipids that trigger ALI via TLR4
- Confirmed histologically (OxPLs) and by assays for ROS, TNF, NFκB and IL-6
- Inactivated H5N1 virus (but not H1N1) produces the same pattern of severe ALI
- Histologic findings in fatal H5N1 influenza in mice and humans are the same

Simvastatin Reduces BALF Inflammation After Inhaled LPS and in Ventilated Patients

- Simvastatin (40 or 80 mg) pretreatment in 20 healthy subjects with 10 controls
- LPS 50 µg inhaled, BALF sampled 6 hrs later
- Simvastatin →↓ neutrophils, MPO, neutrophil elastase, TNFα, IL-1, MMP 7-9 and NF-kappaB
  Shyamsundar M et al. AJRCCM 2009; 179: 1107-14.

- RCT of simvastatin (80 mg qd) in 60 ventilated patients →↓ SOFA scores on d 14 (p = 0.01) and BALF IL-8 (p = 0.05) and IL-6 (p = 0.07)
- Clinical outcomes the same

Craig TR et al. AJRCCM 2011; 183: 620-6.
Pro-resolution Lipoxin A-4 is Down-regulated in H5N1 Influenza

- Mice infected with H5N1 (VN/1203) and 1918 viruses → ↓ lung Alox5 (lipoxin A4)
- H5N1-infected mice died sooner than 1918-infected mice, but virus levels were similar

These results “suggest new lines of experimentation …including specific targeting of the Alox5 gene”

Lovastatin decreases acute airway mucosal inflammation via 15-epi-lipoxin A4

- Murine model of acid-induced airway mucosal injury
- Lovastatin → ↑↑ 15-epi-lipoxin A4
- Lovastatin → ↓↓ acute lung inflammation → ↓↓ PMNs in BALF
- Anti-inflammatory effects of lovastatin and 15-epi-lipoxin A4 administered together were additive

Selective Blockade of Endothelial NF-kappaB Differentially Affects Systemic Inflammation and Multi-organ Failure in Sepsis

- **TG mice** conditionally overexpress IkBα (NFκB inhibitor) selectively on endothelium

- **WT and TG mice** infected i.p with *E. coli*

- **WT mice** → ↑ pro-inflammatory cytokines, ↑ endothelial leakage in lungs, heart, liver and kidney, ↑ multi-organ failure and ↑ mortality

- **TG mice** → blockade of endothelial NFκB had no effect on inflammatory cytokines, but ↓ ICAM-1 and VCAM-1 (markers of endothelial inflammation) in all 4 organs, ↓ endothelial leakage, ↓ MOF, and ↓ mortality

Endothelium is a target, not a source of systemic inflammation

Restoring Endothelial Barrier Integrity Alone Improves Survival in Mice with H5N1 Influenza

Slit2N ↑ VE-cadherin → ↓ VEGF-induced vascular permeability via Robo4, Rho GTPases

• In H5N1-infected BALB/c mice (n=20), Slit2N treatment minimally reduced pulmonary inflammation, but little change in TNFα, IL-1α/β, IFNγ, IL-6, MCP-1, IL-10 and CCR5

• Slit2N → no effect on lung H5N1 virus titers → increased survival

Restoring vascular barrier integrity alone was sufficient to improve survival

Rho kinase inhibition of Robo4 might explain improved survival

Pitavastatin Preserves Endothelial Barrier Integrity in CLP-induced Acute Lung Injury in Mice

- CLP-induced sepsis in BALB/c mice
- Pitavastatin pre-treatment for 4 days and continuing after CLP-induced ALI
- Pitavastatin →↓ hypoxemia, ↓ NF-kappaB, ↓ inflammation, ↓ pulmonary vascular leak

Survival improved from ~30% to ~80%

Mitochondrial Biogenesis and Recovery in Experimental Sepsis and Acute Lung Injury

In mice with untreated sublethal *S. aureus* peritonitis, survival occurs only when there is early mitochondrial biogenesis.

Mitochondrial Biogenesis and Survival in Patients with Critical Illness

- Muscle biopsies were obtained in 10 control patients, 10 with critical illness who survived, and 6 with critical illness who died.

- Critically ill patients who survived had higher levels of mitochondrial respiratory protein subunits (complexes I and IV) and PGC-1α mRNA → early mitochondrial biogenesis.

Mitochondrial Biogenesis and Recovery in Experimental Sepsis and Acute Lung Injury

- In neutrophils, the AMPK agonist metformin inhibits mitochondrial electron transport complex I →
  - ↑ mitochondrial superoxide, $\text{H}_2\text{O}_2$
  - ↓ cytoplasmic LPS-induced $\text{I}\kappa\text{B}\alpha$ degradation
  - ↓ NF$\kappa$B nuclear translocation
  - ↓ TNF$\alpha$, MIP-2

- In LPS-induced ALI in mice, metformin →
  - ↓ MPO, BALF neutrophils, TNF$\alpha$, IL-6 and MIP-2
  - ↓ acute lung injury

Fenofibrate Treatment of Patients with Severe Burn Injury

- Randomized controlled trial in 21 children with extensive burn injury treated for 2 weeks with fenofibrate (5 mg/kg)

- Fenofibrate significantly improved
  - insulin sensitivity
  - cytochrome C oxidase activity
  - mitochondrial glucose oxidation
  - State 3-coupled muscle mitochondrial maximal pyruvate oxidation

Fenofibrate improved mitochondrial function in critically ill patients

Rosiglitazone “Rolls Back” the Host Response of a “Young Adult” to That of a “Child”

<table>
<thead>
<tr>
<th>Hepatic I/R Injury in Mice</th>
<th>“Child”</th>
<th>“Young Adult”</th>
<th>“Old Adult”</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-5 wks</td>
<td>10-12 wks</td>
<td>9-12 mo.</td>
<td></td>
</tr>
</tbody>
</table>

- **PPARγ Activity**: ++, +, ±
- **PPARγ - Nuclear**: ++, -, -
- **PPARγ - Leak, Cytoplasm**: --, +, ++
- **Autophagy**: ++, +, --
- **Rosiglitazone, Autophagy**: nd, ++, nd

• Rosiglitazone pretreatment decreased hepatic ischemia reperfusion injury in “young adult” mice and improved survival.

• Rosiglitazone effects on autophagy might be PPARγ-independent, and instead work through AMPK.

The same response might be seen in young adults with severe influenza.

Fedson DS. *Influenza Other Respi Virus* 2009; 3: 129-42.
Mechanisms for How Immunomodulatory Treatment of Influenza Might Work

- Statins, glitazones, fibrates and metformin would counteract many of the dysregulated cytokine signaling pathways associated with an ‘altered innate immune rheostat,’ as seen in metabolic syndrome.
- Statins and glitazones would up regulate pro-resolution factors such as lipoxin A4.
- Statins, glitazones, fibrates and metformin would reverse pulmonary endothelial cell dysfunction, restore microvascular barrier integrity.
- Statins, glitazones, fibrates and metformin would promote mitochondrial biogenesis (statins not in skeletal muscle)

- Glitazones (and probably metformin) would “roll back” the damaging host response in young adults to the more benign response of children.

These agents might be better used in combination.

Other agents to consider - ACE inhibitors, ARBs, spironolactone, PDE inhibitors.
Immunomodulatory Treatment of Experimental Influenza in Mice
What Has Been Learned?

• Immunomodulatory agents reduce mortality in influenza virus-infected mice by 30-50%
• Benefits are seen with different influenza viruses (H1N1 [PR-8], H2N2 and H5N1)
• Treatment is effective when started after infection, when clinical illness is evident

These agents have no known antiviral activity, yet in the absence of antiviral agents they have not increased influenza virus replication

Fedson DS. Influenza Other Respi Virus 2009, 3: 129-42.
Animal Research on Immunomodulatory Agents for Influenza: Why Do It and How to Do It

- Manage patients
  - identify a small number of promising agents that bring about phenotypic improvement in outcomes
  - evaluate the most promising agents in randomized controlled trials in patients

- Later explain disease pathogenesis and how agents work

- Important to choose the right animal model (mouse) and virus

Fedson DS. *Influenza Other Respi Virus* 2009; 3: 129-42.
Animal Research on Immunomodulatory Agents for Influenza: *Why Do It and How to Do It*

<table>
<thead>
<tr>
<th>Factor</th>
<th>Humans</th>
<th>Mouse, conventional</th>
<th>Mouse, new models</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genetics</td>
<td>Heterogeneous</td>
<td>Specific strain, cytokine KO</td>
<td>More human-like strain</td>
</tr>
<tr>
<td>Immune status</td>
<td>Usually altered</td>
<td>Immunological homeostasis</td>
<td>Test altered immunity (pregnancy, DI0, ob/ob, db/db, ApoE&lt;sup&gt;-/-&lt;/sup&gt;)</td>
</tr>
<tr>
<td>Age and sex</td>
<td>All ages, both sexes</td>
<td>Usually young female adults</td>
<td>Children vs adults, both sexes</td>
</tr>
<tr>
<td>Virus dose</td>
<td>Usually unknown</td>
<td>High dose</td>
<td>Lower dose</td>
</tr>
<tr>
<td>Virus strain</td>
<td>H1N1, H3N2</td>
<td>Often highly mouse adapted (PR8)</td>
<td>Adapt human viruses (pH1N1, H5N1)</td>
</tr>
</tbody>
</table>
H5N1 Pathogenesis in Genetically Diverse Mice

- 21 inbred mouse strains infected with highly pathogenic H5N1 virus
- 50% mouse lethal dose differed $\geq 5$-fold across the strains
- Dose of $10^4$ EID$_{50}$ arbitrarily chosen to distinguish virus-resistant from virus-sensitive mouse strains

H5N1 Pathogenesis in Genetically Diverse Mice

- Fold increases in lung H5N1 virus titers were lower in resistant than in susceptible mice
- Pro-inflammatory cytokine levels were similar in all groups on Day 1, but on Day 3 they were increased more in susceptible mice

Conclusion - viral load dictates the pathogenic response to infection

H5N1 Pathogenesis in Genetically Diverse Mice

“... the host genetic component ... is primarily influencing viral replication. This ... emphasizes the need to limit virus replication ... and it shows that the hyperinflammatory environment is simply a reflection of more viral genetic material inducing a response.”


Caveats

- very high infecting dose in small numbers of mice in each group
- 21 inbred strains comparable to 21 individual outbred mice (or humans)
- no evaluation of anti-inflammatory or pro-resolution factors
- no evaluation of changes in specific lung cells (e.g., macrophages, leukocytes, endothelial cells)
- no evaluation of cytokine changes in other organs (e.g., liver, spleen)
- ignores studies of KO or immunomodulatory agent-treated mice in which outcomes differ without changes in lung virus titers
- ignores targeted improvement in endothelial barrier integrity → ↑ host survival

without any change in lung H5N1 virus titer
- ignores study in which inactivated H5N1 virus causes fatal lung injury in mice

The story is much more complex!
Influenza pH1N1 (CA/04/09) in BALB/c and DBA/2 Mice: Lung Virus Titers

PB1-F2 wild type (rWT) and mutant (66N and 66S) viruses gave similar results

Influenza pH1N1 (CA/04/09) in BALB/c and DBA/2 Mice: Weight Loss and Survival

- PB1-F2 wild type (rWT) and mutant (66N and 66S) viruses → similar diseases
- DBA/2 mice → more severe illness than BALB/c mice

Statins and PPAR and AMPK Agonists: Possible Clinical Benefits in Treating Severe Influenza

• Reduce pulmonary infiltrates, prevent alveolar flooding, maintain oxygenation
• Prevent excessive vasoconstriction /vasodilatation, stabilize myocardial contractility and improve endothelial and epithelial cell function
• Prevent lymphocyte apoptosis, immunosuppression and secondary bacterial infections
• Restore mitochondrial biogenesis, prevent or reverse multi-organ failure
• Switch the overly aggressive host response of adults to the more benign response of children → restore immunological homeostasis
  “Turn down the heat under a boiling kettle”
  → improve survival

• These agents might also be useful in treating other diseases in which the host response is central - pneumococcal sepsis, cerebral malaria, dengue hemorrhagic fever, Ebola virus infection, …
• They might be effective medical countermeasures against agents of bioterrorism - smallpox, anthrax, plague
Why Is It So Difficult for Influenza Scientists to Think That Immunomodulatory Agents Could Be Used to Treat Influenza?

“The most difficult subjects can be explained to the most slow-witted man if he has not formed any idea of them already; but the simplest thing cannot be made clear to the most intelligent man if he is firmly persuaded that he knows already, without a shadow of doubt, what is laid before him.”

Leo Tolstoy, 1897
Immunomodulatory Treatment of Severe Influenza in the Real World

• During the recent H1N1 pandemic, a “top down” approach with vaccines and antivirals completely failed to meet the needs of ≥ 90% of the world’s people
• A “bottom up” approach using agents that modify the host response might improve survival in patients with severe seasonal and pandemic influenza
• Generic immunomodulatory agents are safe, supplies are huge and they are now produced in many developing countries, including China and India
• An individual treatment course would cost € 0.50-1.00
• Treatment would be available to all people with access to basic health care on the first pandemic day

The Goal of Immunomodulatory Treatment of Severe Influenza

Treating patients with severe influenza with immunomodulatory agents should “nudge” a dysregulated host response back toward a state of self-regulated homeostasis.

This should improve survival.
The Possibility of a Severe Influenza Pandemic Has Not Gone Away

In an experiment published in 1974, co-infection of susceptible turkeys with two influenza viruses, one benign and the other highly pathogenic, generated a new reassortant virus that killed all of the infected birds and all of their contacts → a total population collapse!

Treating the Host Response to Severe Influenza

“To see what is in front of one’s nose needs a constant struggle.”

George Orwell  *Tribune*, March 22, 1946
Thank you!
References


• Fedson DS. Confronting the next influenza pandemic with anti-inflammatory and immunomodulatory agents: why they are needed and how they might work. *Influenza Other Respir Virus* 2009; 3: 129-42.


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pH1N1 Influenza in Pregnant Mice
Viral Burden and Immunopathogenesis

- Latter stages of pregnancy →
  - immunosuppression to maintain viable pregnancy
  - ↑ risk of intracellular infection

- Pregnant BALB/c mice (n=5) infected with pH1N1 (Ca/04/09) virus
- Pregnant mice had much higher mortality than non-pregnant mice
- Pulmonary virus levels were similar in both groups

pH1N1 Influenza in Pregnant Mice
Viral Burden and Immunopathogenesis

- Pregnant pH1N1-infected mice →
  ↑ pulmonary infiltrates
  ↑ macrophages, neutrophils, IL-6, MIP-2, TNF, oxidative stress (RNS)
  ↑ Th2 macrophages (↑ IL-4, IL-13 in BALF)
  ↓ lung repair (epithelial regeneration)
  ~ CD4+ and CD8+ T cells
  ↑ Treg cells in lung, not spleen, MLNs

“therapies which modulate inflammation and lung repair … (may reduce) … severe pH1N1 infection in pregnant women”

The Host Response as Understood by Sepsis Scientists

- Early hyperinflammatory response - Th-1, Th-17; IFNγ, IL-1, IL-6, TNFα
- Late immunosuppression - Th-2, Treg; IL-10, TGF-β, lymphocyte apoptosis, secondary bacterial infection (<50%), multi-organ failure and death
- Resolution and recovery - active process, lipoxin-A4
- In fatal cases of sepsis and influenza, the median duration of illness is 10 days
- Most patients do not die during the early “cytokine storm” but later when they are immunosuppressed

Inability to restore homeostasis → multi-organ failure & death


H5N1 Infection in Cytokine Knockout Mice

- Triple mutant mice deficient in TLR-R1, TLR-R2 and IL-1-R1 infected with H5N1 virus
- TM mice compared with WT mice
  - delayed mortality
  - similar lung virus titers
  - ↓ lung cytokines
  - ↓ lung macrophages, neutrophils
  - similar levels of DCs, CD4+ T cells
  - ↓ CD8+ cells at baseline, but no major increase after infection

Genetic modulation modified clinical disease but had no effect on virus replication

“Treatment with anti-inflammatory drugs has been proposed as a therapeutic options for patients infected with H5N1 viruses; however, preclinical testing in H5N1 virus infection models shows little benefit of these treatments. Moreover, treatments with anti-inflammatory drugs, such as corticosteroids, to combat an exaggerated immune response are far too broad in their effects on the immune system. The development of new, more targeted therapies for H5N1 disease, along with combination antiviral drug treatments, could be effective in reducing acute lung injury and mortality caused by H5N1 virus.”


Three experimental studies mentioned by these investigators were superficial and the immunomodulatory agents included only steroids, but not agents known to be effective from earlier studies.
15d-PGJ$_2$ (but not Rosiglitazone) Protects Mice Against Lethal Influenza

- 15d-PGJ$_2$ is a cyclopentone prostaglandin → ↓ TNF, IL-6, IL-12, iNOS, and chemokines CCL2, CCL3, CCL4, CXCL8, CXCL10
- Inhibits NF-κB, stimulates PPARγ
- C57BL/6 mice infected with H1N1 (PR8) virus, treated with 15d-PGJ$_2$ (d1-7)
- Survival improved → 14% to 79%
- PPARγ antagonist (GW9662) abolished 15d-PGJ$_2$ protection
- PPARγ agonist rosiglitazone was not protective
- Dosage was 5 mg/kg → 12 times less than 60 mg/kg protective dose shown in pretreatment of influenza in mice

Statins, Pneumonia Hospitalization and Mortality in Denmark, 1997-2009

- 70,914 pneumonia cases matched 10:1 with controls, adjusted for demographics, co-morbidities and pre-admission medications
- 53,160 cases in supplementary analysis (2001-2009) also adjusted for five social factors and influenza vaccination

<table>
<thead>
<tr>
<th>Method</th>
<th>% Current statins</th>
<th>Outcome</th>
<th>Adjusted OR (95% CI)</th>
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</thead>
<tbody>
<tr>
<td>Case-control, base</td>
<td>10.2</td>
<td>Hospitalization</td>
<td>0.80 (0.77-0.83)</td>
</tr>
<tr>
<td>Case-control, supp.</td>
<td>13.0</td>
<td>Hospitalization</td>
<td>0.77 (0.74-0.80)</td>
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<tr>
<td>Cohort, base</td>
<td>10.2</td>
<td>30-day mortality</td>
<td>0.73 (0.67-0.79)</td>
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<tr>
<td>Cohort, supp</td>
<td>13.0</td>
<td>30-day mortality</td>
<td>0.76 (0.70-0.83)</td>
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</tbody>
</table>

Statins reduce pneumonia hospitalizations and mortality by ~ 25%

### Statins and Pneumonia Mortality Meta-analysis, 2012

<table>
<thead>
<tr>
<th>Study</th>
<th>Weight</th>
<th>Odds Ratio</th>
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<td>0.14, 0.92</td>
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<tr>
<td>Chalmers JD 2008</td>
<td>0.46</td>
<td>0.25, 0.84</td>
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<tr>
<td>Schienger RG 2007</td>
<td>0.47</td>
<td>0.25, 0.88</td>
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<tr>
<td>Mortensen EM 2008</td>
<td>0.58</td>
<td>0.42, 0.80</td>
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<tr>
<td>Myles PR 2009</td>
<td>0.55</td>
<td>0.34, 0.98</td>
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<tr>
<td>Frost 2007</td>
<td>0.60</td>
<td>0.34, 1.06</td>
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<tr>
<td>Douglas I 2011 (6 months)</td>
<td>0.67</td>
<td>0.49, 0.91</td>
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<tr>
<td>Yende S 2011 (3 months)</td>
<td>0.73</td>
<td>0.47, 1.13</td>
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<tr>
<td>Majumdar SR 2006</td>
<td>0.75</td>
<td>0.49, 1.14</td>
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<tr>
<td>Thomsen RW 2008</td>
<td>0.75</td>
<td>0.65, 0.86</td>
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<tr>
<td>Michael B. Rothberg 2011</td>
<td>0.86</td>
<td>0.79, 0.93</td>
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<tr>
<td>Kwong JC 2009</td>
<td>0.91</td>
<td>0.88, 0.94</td>
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<tr>
<td>Subtotal (95% CI)</td>
<td>0.74</td>
<td>0.66, 0.82</td>
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</tbody>
</table>

**Heterogeneity:** Tau^2 = 0.02; Chi^2 = 36.22, df = 11 (P = 0.0002); I^2 = 70%

**Test for overall effect:** Z = 5.27 (P < 0.00001)

**Total (95% CI):**

**Heterogeneity:** Tau^2 = 0.04; Chi^2 = 126.47, df = 34 (P < 0.00001); I^2 = 73%

**Test for overall effect:** Z = 6.68 (P < 0.00001)

**Test for subgroup differences:** Chi^2 = 4.07, df = 3 (P = 0.25), I^2 = 26.2%. Favours experimental