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Management of Pandemic Influenza In 'Have Not' Countries

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The Pandemic Threat and Prospects for H5N1 Vaccination

- Inactivated adjuvanted vaccines
  - development slow, funding limited
  - production capacity limited - 9 countries
  - ~ 700 M people vaccinated with 2 doses in 6 months
  - stockpiling pre-pandemic vaccines possible in only a few countries
- Live-attenuated and recombinant HA vaccines
  - R&D slow and production problematic

Technical limitations are significant, but
organizational, logistical and political limitations are even greater!

Fedson DS, Dunnill P. *J Public Health Policy* 2007; 28: 322-40.

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Tamiflu Treatment of Clade 2 H5N1 Influenza in Indonesia

Interval between onset of illness and treatment	No. of cases	No. of deaths	Case fatality rate
≤ 24 hours	2	0	0 %
0 - 4 days	11	5	45 %
0 - 6 days	37	24	65 %
> 6 days	49	40	82 %
Any treatment	86	64	74 %
NO TREATMENT	*33*	*33*	*100 %*
All cases	119	97	82 %

ISRVI, Singapore, 3 March 2008

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Confronting the Next Pandemic: Could We Use Something Other Than Vaccines and Antivirals?

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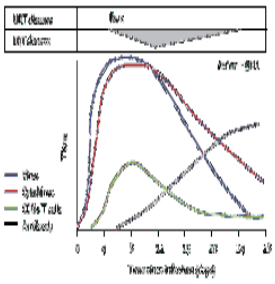
Course of Human Infection with Highly Pathogenic H5N1 Virus



- ↑↑ Pro-inflammatory cytokines in human macrophages and alveolar epithelial cells

  Cheung CY et al. *Lancet* 2002; 360: 1131-7; Chen MC et al. *Respir Res* 2005; 6: 135.
- ↓ IFN-1 α/β in bronchial epithelial cells

  Zeng H et al. *J Virol* 2007: 81: 12439-49.

Gambotto A et al. *Lancet* 2008; 371: 1464-75.

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Hypercytokinemia in Fatal Cases of H5N1 Infection

- Fatal cases had higher levels of pro-inflammatory cytokines

  "Although immunomodulatory treatment has potential benefits at this stage, the focus of clinical management should be on preventing the intense cytokine response by *early diagnosis and effective antiviral treatment*."
- All cases were *diagnosed late*; average 6 days (4-8)
- All but one of 18 cases were *treated with antivirals*, yet 13 *(72%) died!*

de Jong MD et al. *Nat Med* 2006; 12: 1203-7.

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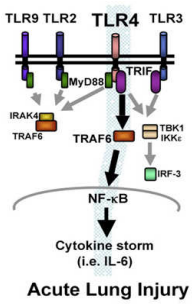
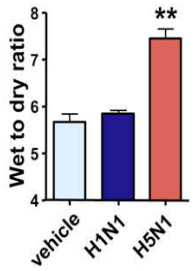
Cell Signaling Pathways in Acute Lung Injury (ALI)



- In acid- and LPS-induced ALI, cell signaling proceeds from TLR4 to NFκB
- Local generation of ROS → oxidized phospholipids that trigger ALI via TLR4

Imai Y et al. Cell 2008; 133: 1-15.

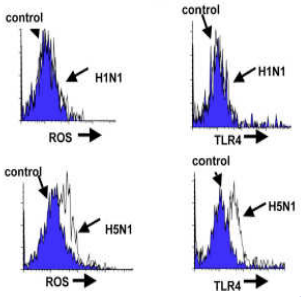
Inactivated H5N1 Influenza Virus Induces Acute Lung Injury



- Inactivated H1N1 and H5N1 viruses instilled intratracheally in C57BL/6N mice
- Lung edema 5 hours later ↑ only in H5N1-treated mice
- Inflammatory response to H5N1 confirmed histologically (NP and OxPLs) and by assays for ROS, NF_kB, IL-6

Imai Y et al. *Cell* 2008; 133: 1-15.

ROS and TLR4 Expression in Murine Alveolar Macrophages After *Inactivated* H1N1 and H5N1 Virus



H5N1 but not H1N1 ↑ formation of reactive oxygen species (ROS) and ↑TLR4

The same changes occur in human PBMCs

Imai Y et al. *Cell* 2008; 133: 1-15.

Heme Oxygenase (HO)-1 Inhibits TLR4 Signaling by Regulating TLR Trafficking to Lipid Rafts

- Ligand-mediated receptor trafficking to lipid rafts is an early event in signal initiation of immune cells
- LPS trafficking of TLR4 to lipid rafts is NADPH oxidase- and RAS-dependent
- ↑HO-1→ ↑CO → ↓LPS-induced NADPH oxidase → ↓ ROS →↓ TLR4 translocation to lipid rafts

↓TLR4 signaling →↓NFκB activation → ↓pro-inflammatory cytokines

Nakahira K et al. *J Exp Med* 2006; 203: 2377-89.

Two Ways to Respond to the Pandemic Threat

Top-down approach - pandemic vaccines and antivirals

- involves only scientific, company and governmental elites
- slow, complex and difficult to organize and manage

'Bottom-up' approach

- uses ordinary people and existing health care systems
- uses abundant supplies of inexpensive generic agents
- would be available worldwide on the first day of a pandemic

The Rationale for a Bottom-up Approach Initially Suggested by Statins

- Statins have anti-inflammatory and immunomodulatory (pleiotropic) effects
- Statins are used to prevent and treat patients at risk of heart attacks, congestive heart failure and strokes
- Influenza is associated with these events
- Influenza vaccination prevents these events
- Influenza increases levels of pro-inflammatory cytokines and chemokines (e.g., TNFα, IL-6)
- Statins decrease them
- *Statins decrease mortality in patients with bacteremia, sepsis and severe pneumonia, illnesses associated with cytokine dysregulation*

Fedson DS. *Clin Infect Dis* 2006; 43: 199-205.

Immunomodulatory Agents That Should Be Considered for Pandemic Use

- Statins – HMG-CoA inhibitors
  - ↓HDL cholesterol and prevent cardiovascular and cerebrovascular disease
  - anti-inflammatory (pleiotropic) effects
- PPARα* agonists (fibrates)
  - regulate lipid metabolism, fatty acid oxidation
  - anti-inflammatory and immunomodulatory effects
- PPARγ agonists (glitazones)
  - increase sensitivity to insulin
  - anti-inflammatory and immunomodulatory effects

* Peroxisome proliferator-activated receptor

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Experimental Acute Lung Injury and the Effects of Statins and PPAR Agonists

Cell signaling molecules	ALI	Statins	PPARα Fibrates	PPARγ Glitazones
TLR4	↑	↓	↓	↓
NFκB	↑	↓	↓	↓
IL-6	↑	↓	↓	↓
ROS	↑	↓	↓	↓
HO-1	(↓)	↑	↑	↑

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Influenza Immunopathogenesis and the Effects of Statins and PPAR Agonists

Cytokines/ chemokines	Influenza	Statins	PPARα Fibrates	PPARγ Glitazones
TNF-α	↑	↓	↓	↓
IL-1	↑	↓	↓	↓
IFN-γ	↑	↓	↓	↓
MIP-1β	↑	↓	n.d.	↓
RANTES (CCL5)	↑	↓	↓	↓
IL-8	↑	↓	↓	↓
MIG	↑	↓	n.d.	n.d.
IP-10	↑	↓	↓	↓

Adapted from La Gruta NL et al. *Immunol Cell Biol* 2007; 85: 85-92.

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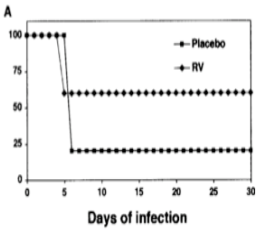
Resveratrol (RES) Improves Survival in PR8-infected Mice

Balb/c mice treated 7d with RES
virus replication ↓■ 2 logs
mortality ↓■ 40%
no anti-oxidant effect (GSH)
↓ nuclear-cytoplasmic translocation of VNPs
↓activity of cellular protein kinases (MAPK, JNK, not ERK 1/2)
Other non-antiviral effects



Palamara A et al. *J Infect Dis* 2005; 191: 1719-29.

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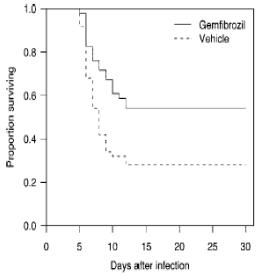
PPARα Agonist Gemfibrozil Improves Survival in Mice with H2N2 Influenza



BALB/c mice - 50 controls, 46 treated with gemfibrozil 60 µg on days 4-10

Mortality - controls 74%
- gemfibozil 48%

Hazard function 0.46
(95% CI 0.26-0.76)

Gemfibrozil significantly reduced H2N2 mortality

Budd A et al. *Antimicrob Agents Chemother* 2007; 51: 2965-8.

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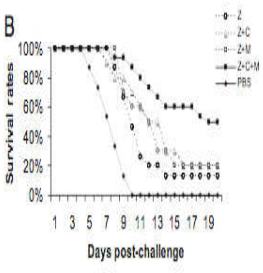
Delayed Antiviral and Immunomodulatory Treatment Increases Survival in H5N1-infected Mice

Balb/c mice → 1000 LD50 A/VN/1194/04 (H5N1)
Treatment (ip) @ 48 hx 6 d
Zanamivir
Z + celecoxib (COX-2 inhib)
Z + mesalazine (PPARγ agonist)
Z + C + M
no treatment (no C + M only)

- Z → ↓ viral load but cytokines and mortality similar to no Rx
- Z + C + M → viral load similar to Z only, but ↓ inflammatory cytokines (p< 0.01) and ↓ mortality (p= 0.02)



Zheng BJ et al. *Proc Natl Acad Sci USA* 2008; 105: 8091-6.

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COX-2-triggered Inflammatory Cascade in Humans and Mice

- In cell culture, H5N1 virus (and LPS) → ↑COX-2 and inflammatory cytokines in macrophages but not in alveolar epithelial (A549) cells
- Supernatants from H5N1-infected macrophages → A549 cells → ↑ COX-2 and inflammatory cytokines
- COX-2 inhibitor treatment of macrophages exposed to H5N1 virus → no increase in COX-2 and cytokines in A549 cells

Interpretation

COX-2 maintains the pro-inflammatory cascade after a decrease in H5N1 replication via a "complex positive feedback loop" and may be a treatment target

Lee SMY et al. *J Infect Dis* 2008; 198: 525-35.

Epithelial Cell Inflammation in ALI is Followed by COX-2-dependent Active Resolution

- Early ALI - ↑ COX-2 → ↑ LTB4, PI3K, ROS, TNFα, ↑ NADPH oxidase, ↑ edema
- COX-2-derived PGD2 (LTB4) switches to PGE4 → ↑ lipoxins (LX), especially LXA4
- LX - ↓ TNFα, IL-6 and ROS,↓ pmn superoxide and chemotaxis, ↑ macrophage phagocytosis of apoptotic neutrophils, ↓ edema, ↓ VEGF-mediated angiogenesis, ↓ MMPs, ↑ HO-1
- Early COX-2 inhibition → ↓ pmn trafficking
- Late COX-2 inhibition → ↓ LXA4 → ↑ inflammation and prolonged recovery

Fukunaga K et al. *J Immunol* 2005; 174: 5033-9.

Bonnans C et al. *Am J Respir Cell Mol Biol* 2007; 36: 201-5.

Statins Activate PPARγ and PPARα Through COX-2 Expression in Macrophages

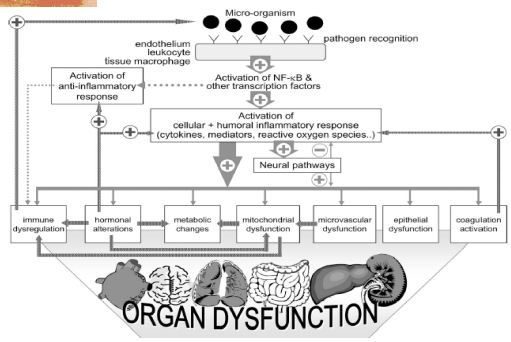
- Statins → ↑ERK1/2 and p38 MAP kinase → ↑ COX-2 expression → ↑ prostaglandin 15d PGJ_2 → ↑ PPARγ and PPARα
- Statins → ↓ TNFα and MCP-1 and these anti-inflammatory effects are inhibited by blocking PPARγ and PPARα

Yano M et al. Circ Res 2007; 100: 1442-51.

Because the anti-inflammatory effects of statins and PPARγ and PPARα agonists are mediated by COX-2, COX-2 inhibition might not always be helpful

Mechanisms of Sepsis-induced Multi-organ Dysfunction

Abraham E, Singer M. *Crit Care Med* 2007; 35: 2408-16.



Statins, PPAR Agonists and Influenza Possible Mechanisms of Action

Direct antiviral effects

- Alter numerous intracellular signaling pathways needed for virus replication
- Alter microdomains on lipid rafts → ↓ virus assembly and budding

Statins, PPAR Agonists and Influenza Possible Mechanisms of Action

Non antiviral effects that improve cardiopulmonary endothelial and epithelial cell function and restore energy homeostasis in all vital organs

- ↓ NF-κB and ↓ AP-1 → ↓ cytokines, chemokines, cellular adhesion molecules; modify caspase activation and apoptosis
- ↑ pro-resolution lipoxin A4, resolvin D1, protectin D1
- ↑ eNOS → ↑ NO,↑ vasodilatation, ↑ cardiovascular function
- Alter actin cytoskeleton and intracellular tight junctions, ↑ lung barrier function, ↓ vascular leak
- ↑ HO-1 → ↓ oxidative stress, ↓TLR4 and downstream signaling
- Restore mitochondrial homeostasis, ↑ energy supply (ATP)

An Epidemiological 'Signal of Protection' Statins Reduced Pneumonia Mortality

- Population-based, nested case-control study of 1227 cases of pneumonia each matched with three controls
- Evaluated effects of statins prescribed during 30 days prior to hospitalization

Statin reduction	Adjusted OR	(95% CI)
pneumonia hospitalization	*0.63*	(0.46 to 0.88)
30-day pneumonia mortality	*0.47*	*(0.25 to 0.88)*

Statins reduced pneumonia mortality by 53%

Schlienger RG et al. *Pharmacotherapy* 2007; 27: 325-32.

Statins Reduce ICU Pneumonia Mortality *Randomized Controlled Trial*

- 67 ICU pneumonia patients randomized to receive atorvastatin 10 mg (33) or placebo (34) qd
- No differences between groups in age, cholesterol (day 1), APACHE II, ALI and SOFA scores and Pneumonia Severity Index
- Cholesterol lower in statin group on day 7
  90 vs. 118 mg/dl; p = 0.044

	% Mortality Controls	Statin	% reduction	p value
ICU	50	27.3	45.4	0.08
Hospital	55.9	27.3	*51.2*	*0.026*

Choi et al. *Crit Care Med* 2007; 35: (12 Suppl): A15.

Other Agents for Pandemic Treatment and Prophylaxis

- *Consider any available and inexpensive generic anti-inflammatory, immunomodulatory or antiviral agent*
  - COX-2 inhibitors
  - ACE inhibitors – ↓ ACE gene expression
  - angiotensin-II receptor blockers (ARBs)
  - spironolactone, phosphodiesterase inhibitors
  - resveratrol and chloroquine (both have anti-influenza activity)
  - naltrexone, metformin, ethyl pyruvate, curcumin → ↓ NF-kappa-B
  - bupropion → ↓ TNF-α, IFN-γ and IL-1β, ↑ IL-10
- If effective, these agents could be used alone, with antivirals, or as combination therapy with statins, PPAR agonists and/or other agents

Could Generic Agents Be Used for Pandemic Treatment and Prophylaxis?

- *There is a global need for effective agents to complement limited supplies of vaccines and antivirals*
- Generic agents are *now being produced in developing countries*
  - simvastatin by ~ 102 companies, more than 50% located in China and India
  - fibrates by many companies, some in China and India
- Five-days of treatment in developing countries would be *inexpensive*
  - simvastatin → < $1 fibrates → ~ $3-5, probably much less

Fedson DS, Dunnill P. *J Public Health Policy* 2007; 28:322-40.
Fedson DS. *Lancet Infect Dis*, 2008; 8: 571-6.

Five-point Research Agenda for Using Generic Agents in a Pandemic

1. Test candidate treatments in whole animals (mice, ferrets and non human primates) to identify specific agents that might be effective in *managing* an H5N1-like pandemic

2. Later, study promising treatments in cell culture and animals to define the molecular mechanisms that *explain* their beneficial effects in H5N1 virus infection

Who should do this work?

- molecular biologists who know cell signaling in inflammation, the immune response and energy homeostasis in acute lung injury, sepsis and multi-organ failure
- laboratory investigators and clinicians from critical care, cardiovascular and pulmonary diseases, endocrinology and metabolism and pharmacology and therapeutics

This work is far too important to be left to influenza scientists alone!

Five-point Research Agenda for Using Generic Agents in a Pandemic

3. Identify developing countries where these generic agents are produced, determine quantities produced, surge capacities, patterns of distribution and costs

4. Establish a process to manage the stockpiling of generic agents and/or their distribution in a pandemic

5. Conduct randomized controlled trials of promising treatments immediately after the emergence of a new pandemic virus

Fedson DS. The challenge of pandemic preparedness for developing countries: what's missing. To be published.

Clinical Trial of a New Treatment Immediately After the Onset of the Next Pandemic*

Mortality reduction	Total sample size (power) 80%	90%	95%
25%	530	690	850
50%	140	170	210
75%	60	80	90

* Liss D, Fedson DS. Unpublished calculations assume 1:1 randomization of subjects to two treatment groups, untreated mortality = 50% and $\alpha = 0.05$ (two-sided).

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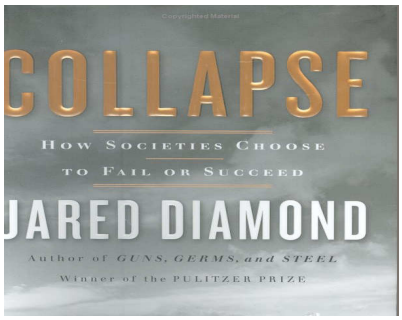
What Will Be The Consequences of the Next Pandemic ?



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How Will We Confront the Next Pandemic?

Generic agents might not work, but
We have a choice

- We can undertake the necessary research *before* the pandemic arrives and show that generic agents will not be effective, or
- We can undertake the research *after* the pandemic has passed and show that millions could have been saved

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Preparing for the Next Pandemic Concluding Thoughts

- The next influenza pandemic might not bring on a global catastophe, but everything we know about influenza virology tells us it could
- For the next five years and probably longer, a "top down" approach using pandemic vaccines and antivirals won't meet the needs of > 85% of the world's people who live in "have not" countries
- A "bottom up" approach the uses inexpensive and widely available generic agents could help meet their needs

For now, a "science for management"
must supercede a "science of explanation"

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Confronting the Next Pandemic With Inexpensive Generic Agents

"It is not enough to say, 'We are doing our best.'
You have got to succeed in doing what is necessary."

Winston Churchill

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References

- Fedson DS. Pandemic influenza: a potential role for statins in treatment and prophylaxis. *Clin Infect Dis* 2006; 43: 199-205.
- Fedson DS, Dunnill P. From scarcity to abundance: pandemic vaccines and other agents for "have not" countries. *J Public Health Policy* 2007; 28: 322-40.
- Fedson DS. Confronting an influenza pandemic with inexpensive genric agents: can it be done? *Lancet Infect Dis* 2008; 8: 571-6.
- Fedson DS. Meeting the challenge of influenza pandemic preparedness in developing countries. *Emerg Infect Dis* 2009; 15: 369-71.

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