CAN WE MAKE BETTER INFLUENZA VIRUS VACCINES?

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Bangkok, January 25th, 2008
INFLUENZA VIRUSES CIRCULATING IN THE HUMAN POPULATION

A

- H1
- H2
- H3
- H1N1
- H3N2

B

YEAR

1889 1900 1918 1940 1960 1980 2000
INFLUENZA VACCINES

KILLED (INACTIVATED)
FORMALDEHYDE TREATED

LIVE (ATTENUATED)
COLD-ADAPTED DONOR STRAIN
Boosting immunity to influenza H5N1 with MF59-adjuvanted H5N3 A/Duck/Singapore/97 vaccine in a primed human population

Iain Stephenson, Karl G. Nicholson, Anthony Colegate, Audino Podda, John Wood, Ellen Ypma, Maria Zambon

*a* Infectious Diseases Unit, Leicester Royal Infirmary, Leicester, LE1 5WW, UK

*b* Chiron Vaccines, Siena, Italy and Amsterdam, Amsterdam, The Netherlands

*c* National Institute for Biological Standards and Control, Potton Bar, Hertfordshire, UK

*d* Public Health Laboratory Service, Colindale, London, UK

Received 14 May 2002; received in revised form 7 October 2002
Live attenuated influenza virus vaccines

POTENTIAL ADVANTAGES

- Humoral and cellular immunity
- Mucosal immunity
- Long lasting memory
- Crossprotection against variant strains

NIH grants: Live attenuated vaccines for epidemic and pandemic influenza U01AI070469
North East Biodefense Center U54AI057158
Center for Research on Influenza Pathogenesis HHSN266200700010C
REVERSE GENETICS FOR USE IN VACCINES
(plasmid-only)

Viral RNA expression plasmids
- PB2
- PB1
- PA
- HA
- NP
- NA
- M
- NS

Protein expression plasmids
- PB2
- PB1
- PA
- NP

Transfection

Cells

Recombinant influenza virus (6:2 reassortant)
Optimal live attenuated vaccines

Immunogenicity

Attenuation
Influenza A And B Viruses Expressing Altered NS1 Proteins: A Vaccine Approach

JULIE TALON  MIRELLA SALVATORE
ROBERT O’NEILL  YURIE NAKAYA
PETER PALESE  HONGYONG ZHENG
ADOLFO GARCÍA-SASTRE

THOMAS MUSTER

Talon et al., PNAS 97, 4309, 2000
Structure of Influenza A viral NS genes

- PR8 (wt NS)
- NS1-99
- ΔNS1

PNAS 2000, 97, 4309-4314
The NS1 gene prevents induction of IFN in influenza virus infected cells

Xiuyan Wang
delNS1 | NS1-99 | PR/8

no IFN antagonist | intermediate IFN antagonist | strong IFN antagonist

HOST WINS | ATTENUATION | VIRUS WINS
Enhanced stimulatory activity by influenza virus NS1 mutant infected human dendritic cells on T cells

IFNγ ELISA of T-cell co-culture with Flu infected human cDCs

MOI 0.5

MOI 1

NI TXWT TX99 TX 1-126

IFNγ pg/ml

ANA FERNANDEZ-SESMA
### Survival of mice immunized with NS1 attenuated influenza A viruses

<table>
<thead>
<tr>
<th>Immunizing virus</th>
<th>Survivors following challenge</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/delNS1</td>
<td>9/14</td>
</tr>
<tr>
<td>A/NS1/1-99</td>
<td>12/12</td>
</tr>
<tr>
<td>PBS</td>
<td>0/6</td>
</tr>
</tbody>
</table>

- **Immunize** 4 weeks with PR8immunize
- **Challenge** with PR8
Can equine influenza A virus be attenuated by deletions in the NS1 gene?

Influenza A/eq/KY/02 H3N8
Wild type
NS1/1-126
NS1/1-99
NS1/1-73

Michelle Quinlivan
Dmitriy Zamarin
Adolfo Garcia-Sastre
Peter Palese
Ann Cullinane
Irish Equine Center

Thomas Chambers
University of Kentucky
Vaccination with NS1 mutant (NS1-126)

9 yearling horses
--6 vaccinates
--3 unvaccinated controls

Virus administered by intranasal route, $3 \times 10^7$ EID$_{50}$U/horse
PROTECTION OF HORSES AGAINST H3N8 VIRUS CHALLENGE FOLLOWING VACCINATION WITH NS1-126 MUTANT

Clinical Score

Days Post Infection

-4 -3 -2 -1 0 1 2 3 4 5 6 7 8

Rx

Vaccinates
Controls
Can primary isolates of influenza A virus be attenuated by deletions in the NS1 gene?

Influenza A/sw/Texas/98
H3N2
Wild type
NS1/1-126
NS1/1-99
NS1/1-73

Alicia Solórzano
Adolfo Garcia-Sastre

Jürgen Richt, Kelly M. Lager
USDA, Ames, Iowa

Bruce H. Janke
Iowa State University

Richard Webby
St. Jude Children's Research Hospital
CONCLUSIONS (I)

Reverse genetics techniques can be used to generate NS1 mutant influenza viruses.

Influenza viruses containing truncations in their NS1 genes are attenuated and immunogenic in four animal systems (mice, horses, pigs and macaques). By virtue of their enhanced ability to activate DCs, they are self-adjuvanted vaccines.

NS1-truncated influenza viruses represent a new generation of live attenuated influenza virus vaccines.
AVIAN INFLUENZA IS A THREAT
Engineered influenza virus vaccine constructs with dual specificity: Avian influenza and Newcastle disease

Rescue of an attenuated chimeric influenza A/VN/1203/04 virus expressing NDV hemagglutinin/neuraminidase

A Combination *in-ovo* Vaccine against Avian Influenza and Newcastle Disease

The recombinant bivalent influenza VN/HN virus derives 7 gene segments from Influenza A/VN/1203/04 virus (H5N1) and the HN gene from the Newcastle disease Hitchner/B1 strain.
Incorporation of viral segments into virion

Expression of HA and NA-HN proteins in infected cells

Timeline of vaccination experiment

Day 0

In-ovo vaccination of 18-day-old chicken embryos with $10^6$ EID$_{50}$ of bivalent influenza VN/HN virus

Day 21

1. Sera collected

2. Challenge with $10^6$ EID$_{50}$ of influenza A/Vietnam/1203/04 virus or $10^5$ EID$_{50}$ of NDV Fontana virus

Day 35

Sera collected
Protection of 3-week-old chickens challenged with highly pathogenic influenza A/VN/1203/04 or highly virulent NDV Fontana following in-ovo vaccination with bivalent VN/HN virus

<table>
<thead>
<tr>
<th>Bird #’s</th>
<th>Vaccine Group</th>
<th>Challenge Group</th>
<th>Morbidity</th>
<th>Mortality (MDT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-10</td>
<td>VN/HN</td>
<td>NDV Fontana</td>
<td>1/10</td>
<td>1/10 (6.0)</td>
</tr>
<tr>
<td>11-20</td>
<td>VN/HN</td>
<td>Influenza VN/1203/04</td>
<td>2/10</td>
<td>2/10 (2.5)</td>
</tr>
<tr>
<td>21-30</td>
<td>Sham</td>
<td>NDV Fontana</td>
<td>10/10</td>
<td>10/10 (5.7)</td>
</tr>
<tr>
<td>31-40</td>
<td>Sham</td>
<td>Influenza VN/1203/04</td>
<td>10/10</td>
<td>10/10 (1.6)</td>
</tr>
</tbody>
</table>
Engineered NDV vaccine constructs with dual specificity: Avian influenza and Newcastle disease

Man-Seong Park, John Steel, Adolfo García-Sastre, David Swayne, and Peter Palese*
Construction of rNDV full length clone containing foreign gene

Gene end/start sequence

H5 Hemagglutinin

Xba I
CONCLUSIONS
Influenza Virus Vaccines

• Improvements for killed as well as live human virus vaccines appear possible (reverse genetics, adjuvants, novel attenuated strains).

• Bivalent avian vaccines against Newcastle disease and highly pathogenic avian influenza can be designed (H5 influenza virus expressing NDV hemagglutinin/neuraminidase; NDV expressing H5 hemagglutinin).
The Guinea Pig as a Transmission Model for Influenza

- Anice Lowen
- Samira Mubareka
- Terry Tumpey (CDC)
- Adolfo García-Sastre
- Peter Palese
Airborne Transmission

For influenza A/Panama/2007/1999 (H3N2) virus the guinea pig infectious dose 50 (GPID50) = 5

In this system there is 100% transmission
Hypothesis:

Aerosol transmission of influenza virus varies with ambient relative humidity and temperature

Approach:

Using the guinea pig transmission model, assess the efficiency of transmission under varying conditions of relative humidity and temperature
Transmission cage

Environmental chamber

EXPOSED INFECTED

EXPOSED INFECTED

EXPOSED INFECTED

EXPOSED INFECTED

AIR FLOW
Aerosol transmission of influenza virus from guinea pig to guinea pig is most efficient at low temperature.
35% Relative Humidity and 20°C

100% transmission
80% Relative Humidity and 20°C

0% transmission
How does influenza virus spread?

• Limited data are available with respect to the relative contributions of different routes of transmission of influenza virus.

• Resolution of this discussion is paramount to the development of effective control measures in healthcare and community settings.

Aim:
To determine the efficiency of influenza virus transmission through different routes of spread.
Indirect Contact (fomite) Transmission of Pan99 in Hartley Guinea Pigs

Results:

Paw inoculation
- 0/4 infected
- 1/4 seroconverted

Environmental application
- 0/6 infected
- 0/6 seroconverted
- 2/2 intranasally-inoculated shed $10^6$ PFU/ml and seroconverted
### INDIRECT CONTACT (FOMITE) TRANSMISSION

Nasal wash alternating days

Acute & terminal bleeds for serology

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<table>
<thead>
<tr>
<th>Inoculated guinea pigs</th>
<th>Exposed guinea pigs</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Expt no.</strong></td>
<td><strong>Peak nasal wash titer (PFU/ml) (n)</strong></td>
</tr>
<tr>
<td>------------------------</td>
<td>-------------------------------</td>
</tr>
<tr>
<td>1 Hartley</td>
<td>4.4±4.2x10^6 (8)</td>
</tr>
<tr>
<td>2 Hartley</td>
<td>3.5±1.3 x10^6 (4)</td>
</tr>
<tr>
<td>3 Strain 13</td>
<td>3.3±0.16 x10^6 (4)</td>
</tr>
</tbody>
</table>
Air Sampling of Exhaled Breaths of Infected Guinea Pigs

Air inlet

Air flow = 12.5 L/min

Lid sealed with gasket & clamps

Air sampling outlet

<table>
<thead>
<tr>
<th>Duration of air sampling (min)</th>
<th>Collection Volume (ml)</th>
<th>Titers (PFU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Experiment #1: T=10°C, RH=60%</td>
</tr>
<tr>
<td>10</td>
<td>5.0</td>
<td>220</td>
</tr>
<tr>
<td>10</td>
<td>10.0</td>
<td>520</td>
</tr>
<tr>
<td>20</td>
<td>5.0</td>
<td>1560</td>
</tr>
<tr>
<td>20</td>
<td>10.0</td>
<td>350</td>
</tr>
</tbody>
</table>
• Transmission via fomites is inefficient compared to respiratory droplet spread.

• Infected guinea pigs shed viable influenza virus into the air.

• Transmission in an upward direction over a distance >1m suggests that droplet nuclei transmission occurs.