Vaccine prevention of non-influenza viral respiratory disease: current and future.

ESCMID 10-13 April 2010
Vienna, Austria
Human respiratory tract disease
- Associated pathogens -

540 respiratory GP patients, Netherlands, 1996-1997, culture and PCR (NIVEL)

- Bacteria, 16%
- Mycoplasma pneumoniae, 1%
- Chlamydia pneumoniae, 1%
- Parainfluenza virus, 1%
- Adenovirus, 1%
- Coronavirus OC43, 2%
- Enterovirus, 4%
- RSV, 5%
- Influenza B virus, 9%
- Influenza A (H3N2) virus, 14%
- Rhinovirus, 22%
- Negative, 36%
Virus discovery
- expertise needed -

- Clinical diagnosis
- Pathology
- Epidemiology
- Laboratory
  - classical virology
  - electron microscopy
  - serology
  - animal models
  - molecular biology
Online Lecture Library

Slide withheld at request of author
Newly identified human respiratory viruses in last 15 years alone

- AIV`s * influenza virus 1997...
- Hendra-/NipahV paramyxovirus 2000...
- hMPV * paramyxovirus 2001
- SARS-CoV * coronavirus 2003
- HCoV-NL63 * coronavirus 2004
- HCoV-HKU1 coronavirus 2005
- HBoV parvovirus 2005
- KI/WU-PyV polyomavirus 2007
- MeIV (KamV) orthoreovirus 2007 (2009)
- H1N1v influenza virus 2009
- ...

  - animal origin  * ErasmusMC involvement
Role of human metapneumovirus, human coronavirus NL63 and human bocavirus in infants and young children with acute wheezing

Fig. 2. Seasonal distribution of (A) hMPV, (B) HCoV NL63, (C) HBoV, (D) other viruses in children with acute wheezing over a 19-month period, including two winter seasons.
Order Mononegavirales, family Paramyxoviridae

Human metapneumovirus
- Rivers’ modified Koch’s postulates -

1. Virus isolation
2. Virus propagation
3. Filtration

0.2 / 0.4 μM

4. Disease in macaques
5. Re-isolation & PCR of virus
6. Specific immune response

Disease in macaques

Specific immune response

OA, 1gA, 1gM, 1gG
Newly discovered human paramyxovirus hMPV
- Risk groups -

- (Young) children
  ~10% of children with RTI

- Immunocompromised individuals (fatal cases!)

- Elderly

- Normal individuals
  ~5% of RTI in community surveillance studies

Osterhaus and Fouchier, Lancet 2003
v.d. Hoogen et al., JID 2003
## Human metapneumovirus
- Vaccine approaches -

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Animal model</th>
<th>Authors</th>
</tr>
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<td>Soluble F protein</td>
<td>Hamsters, cynomolgus macaques</td>
<td>Herfst, 2007/2008</td>
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<tr>
<td>Soluble F protein and F DNA vaccine</td>
<td>Cotton rats</td>
<td>Cseke, 2007</td>
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<tr>
<td>FI-HMPV</td>
<td>Macaques, cotton rats</td>
<td>De Swart, 2007</td>
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<td>Yim, 2007</td>
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<td>HI-HMPV</td>
<td>Mice</td>
<td>Hamelin, 2007</td>
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<td>Cpts HMPV</td>
<td>Hamsters, cynomolgus macaques</td>
<td>Herfst, 2008</td>
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<tr>
<td>HMPV deletion mutants</td>
<td>Hamsters, AGM</td>
<td>Biacchesi, 2004/2005</td>
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<td></td>
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<td>Buchholz, 2005</td>
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<td>Chimeric HMPV / AMPV-C</td>
<td>Hamsters, AGM</td>
<td>Pham, 2005</td>
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<tr>
<td>B/HPIV3 expressing F</td>
<td>Hamsters, AGM</td>
<td>Tang, 2003/2005</td>
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<tr>
<td>Alphavirus replicon particles expressing F</td>
<td>Mice, cotton rats</td>
<td>Mok, 2008</td>
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<tr>
<td>T-lymphocyte vaccine</td>
<td>Mice</td>
<td>Herd, 2006</td>
</tr>
</tbody>
</table>
b/h PIV3 expressing hMPV F gene

Avrll 104 
Avrll 1774

b/h PIV3

F N P/C M hPIV3 F hPIV3 HN L

b/h hMPV F1

F N P/C M hPIV3 F hPIV3 HN L

b/h hMPV F2

N F P/C M hPIV3 F hPIV3 HN L


MedImmune, Inc
**b/hPIV3 expressing hMPV F gene**

Hamsters immunized with bovine/human PIV3 expressing hMPV F protein are protected from challenge with hMPV or hPIV3.

<table>
<thead>
<tr>
<th>Challenge virus:</th>
<th><strong>hPIV3</strong></th>
<th><strong>hMPV</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean virus titer on day 4 post-challenge (log&lt;sub&gt;10&lt;/sub&gt; TCID&lt;sub&gt;50&lt;/sub&gt; g tissue ± S.E.)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Mean virus titer on day 4 post-challenge (log&lt;sub&gt;10&lt;/sub&gt; pfu/g tissue ± S.E.)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Immunizing Virus&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Nasal Turbinates</td>
<td>Lungs</td>
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<tr>
<td>-----------------</td>
<td>----------------</td>
<td>------</td>
</tr>
<tr>
<td>b/h PIV3</td>
<td>&lt;1.3 ± 0.2</td>
<td>&lt;1.1 ± 0.1</td>
</tr>
<tr>
<td>b/h hMPV F1</td>
<td>&lt;1.3 ± 0.1</td>
<td>&lt;1.1 ± 0.1</td>
</tr>
<tr>
<td>b/h hMPV F2</td>
<td>&lt;1.2 ± 0.1</td>
<td>&lt;1.2 ± 0.1</td>
</tr>
<tr>
<td>hMPV</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>placebo</td>
<td>4.3 ± 0.3</td>
<td>4.5 ± 0.5</td>
</tr>
</tbody>
</table>

<sup>a</sup> Virus used to immunize groups of six hamsters on Day 0.

<sup>b</sup> On Day 28, the hamsters were challenged with 10<sup>6</sup> pfu of hPIV3 or hMPV/NT/1/00.

ND = not determined.

MedImmune, Inc
F subunit vaccine
- Immunization / challenge -

-Vaccines:
* F1/00 (A1) Specol
* F1/00 Iscom
* F1/00 nonAdj
* F1/99 (B1) Specol
* F1/99 Iscom
* F1/99 nonAdj
* Specol
* Iscom
* PBS

-Hamsters were immunized twice, 3 week interval (10ug F)
-Challenge with 10^6 TCID50 of recNL/1/00 (A1)
-Lungs and nasal turbinates were collected 4 dpi
F subunit vaccine
- Immunization / challenge -

Herrst et al., 2007/2008
F subunit vaccine
- Immunization / challenge -

Herfst et al., 2007/2008

virus titers in lungs
Cold-passaged viruses
- cpNL/1/99 (B1) -

- NL/1/99 (B1) was passaged at gradually decreasing temperatures (25 °C), pass 35 was sequenced
- Recombinant virus (HMPV11) was cloned and rescued

- Four ts RSV mutations were found that can be introduced in recombinant HMPV
- Recombinant virus (NL/1/99 (B1) backbone) containing 3 mutations was cloned and rescued (HMPV/R3)
  - Shut-off temperature $\geq 38$ °C in vitro
  - Viruses attenuated in hamsters
Immunization of macaques
- F subunit / cptsHMPV11 -

- t=0  first imm, 10μg Fsol (Iscom) / 10^6 TCID_{50} HMPV11 / PBS
- t=28  second imm.
- t=84  heterologous challenge 10^{6.5} TCID_{50}
- 1, 3, 5, 9 dpi collection of throat swabs / BAL samples
Immunization of macaques

- PRVN -

Herfst et al., 2007/2008

1/99 (B1) Homol.

1/00 (A1) Heterol.
Cold-passaged viruses
- Results challenge infection -

Herfst et al., 2007/2008

Immunization: $10^6$ TCID$_{50}$
Challenge: $10^7$ TCID$_{50}$
Paramyxovirus in SARS patients

Hong Kong researchers announce findings of Paramyxoviruses in SARS patients. Similar findings communicated from Canada through SARS etiology network.
Cynomolgus macaques
(Macaca fascicularis)

Simultaneous SCV-hMPV Infection-experiments:

- SCV
- hMPV
- SCV followed by hMPV

Fouchier et al., Nature 2003
Kuiken et al., Lancet 2004
Gross pathology in aged macaques

3-5 yrs young

10-20 yrs aged

Gross pathology score (%)

[Graph showing comparison between young-adult and aged groups]

[Table or heatmap showing differential gene expression]

de Lang et al., PLoS Path. 2007
Smits et al., Plos Path. 2010
Administration of pegylated IFN-alpha in aged macaques

Haagmans et al., Nature Med. 2004
Smits et al., Plos Path. 2010
Possible problems with “a candidate SARS vaccine”

- Lack of efficacy
  -- problems with animal coronavirus vaccines!

- Safety concerns
  -- antibody mediated enhancement (AME)
    example: FIP candidate vaccines!
  -- predisposition for more serious disease
    examples: i.a. RSV- and measles vaccines!
Vaccine-induced enhancement of viral infections

Table 1
Mechanisms of enhancement of susceptibility to virus infection or of aberrant viral pathogenesis mediated by pre-existing immunity.

<table>
<thead>
<tr>
<th>Mechanisms</th>
<th>Virus families</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Flaviviridae</td>
</tr>
<tr>
<td>Humoral ADE</td>
<td>DENV</td>
</tr>
<tr>
<td></td>
<td>WNV</td>
</tr>
<tr>
<td></td>
<td>MVEV*</td>
</tr>
<tr>
<td>Cellular CD4 activation</td>
<td></td>
</tr>
<tr>
<td>DC/trans Aberrant T-cell response</td>
<td>MV (?)</td>
</tr>
</tbody>
</table>

(?) = mechanism unknown/ambiguous. Abbreviations—ADE: antibody-dependent enhancement; DENV: Dengue virus; WNV: West Nile virus; MVEV: Murray Valley encephalitis virus; FCoV: Feline Corona virus; MV: measles virus; RSV: respiratory syncytial virus; HMPV: human metapneumovirus; HIV: human immunodeficiency virus; SIV: simian immunodeficiency virus; FIV: feline immunodeficiency virus; EIAV: equine infectious anaemia virus; DC: dendritic cells.

* in vitro.
Antibody mediated enhancement in FIP

• Hallmarks:
  - faster onset of disease
  - more fulminant course of disease

• Hypothesis:
  - *S*-specific antibodies mediate infection of macrophages
Inactivated paramyxovirus vaccines: lessons for SARS?

- 1960’s: development of **inactivated RSV** and **MV** vaccines: **formaldehyde**-inactivated whole virus preparations (FI-RSV / FI-MV) precipitated with **aluminium** phosphate / hydroxide

- Vaccination induced **short-lived antibody** and **inbalanced T cell** responses

- Vaccination predisposed infants for **enhanced disease** following subsequent natural infection with the respective viruses

→ **Immunopathology !!!!!**
Adverse effects paramyxovirus vaccines

- Formalin-inactivated paramyxovirus vaccines adjuvanted with alum can predispose to hypersensitivity responses

- Similar pathology seen for FI-RSV, FI-MV, and FI-hMPV

- Differences:
  - FI-RSV study: mild eosinophilic tracheobronchitis, but two fatal cases (with low eosinophil counts!)
  - FI-MV study: severe eosinophilic tracheobronchitis
  - FI-hMPV study: eosinophilic alveolitis
Two FI-RSV animals died with hyperinflation.

- **Histopathology:** no abnormalities
- **In vitro correlates:** high IL-13 and IL-5 responders in the absence of detectable IFN-γ responses

Histopathology: infiltration of eosinophils upon challenge after vaccination with experimental FI-RSV vaccine

- **Eosinophilic tracheo-bronchitis**
- **Eosinophilic bronchiolitis**
- **Diffuse eosinophilic alveolitis**

*Blue: control animals  Red: FI-RSV primed animals*

# Candidate SARS Vaccines

<table>
<thead>
<tr>
<th>Developer</th>
<th>Type</th>
<th>Funding</th>
<th>Location</th>
<th>Human trials, target</th>
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</thead>
<tbody>
<tr>
<td>Sinovac/CAMS</td>
<td>Inactivated virus</td>
<td>China</td>
<td>China</td>
<td>March 2004</td>
</tr>
<tr>
<td>Univ. of British Columbia</td>
<td>Inactivated virus</td>
<td>Canada</td>
<td>Canada</td>
<td>December 2004*</td>
</tr>
<tr>
<td>Univ. of Toronto</td>
<td>Recombinant</td>
<td>Canada</td>
<td>Canada</td>
<td>December 2004*</td>
</tr>
<tr>
<td>McMaster Univ.</td>
<td>Adenovirus</td>
<td>Canada</td>
<td>Canada</td>
<td>December 2004*</td>
</tr>
<tr>
<td>Aventis Pasteur</td>
<td>Inactivated virus</td>
<td>NIAID contract</td>
<td>France</td>
<td>Late 2005</td>
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<tr>
<td>Baxter Healthcare</td>
<td>Inactivated virus</td>
<td>NIAID contract</td>
<td>Austria</td>
<td>Late 2005</td>
</tr>
<tr>
<td>Protein Sciences</td>
<td>Recombinant</td>
<td>NIAID contract</td>
<td>U.S.</td>
<td>Late 2005</td>
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<tr>
<td>U.S. Vaccine Research Center</td>
<td>Plasmid DNA</td>
<td>NIAID</td>
<td>U.S.</td>
<td>December 2004</td>
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<tr>
<td>Chiron Vaccines</td>
<td>Inactivated virus</td>
<td>Chiron</td>
<td>Italy</td>
<td>Not set</td>
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<tr>
<td>Univ. of Pittsburgh</td>
<td>Adenovirus</td>
<td>NHLBI/CDC</td>
<td>U.S.</td>
<td>Not set</td>
</tr>
</tbody>
</table>

* Canada will choose one of three candidates for clinical testing after a head-to-head competition in March.
Vaccination of macaques with inactivated SARS CoV (with/without alum)

- BPL inactivated SARS CoV
- In the absence or presence of aluminium hydroxide
- SARS CoV challenge with $10^7$ TCID$_{50}$ SARS CoV

- Immunization
- Challenge

Week: 0 4 19

Necropsies were carried out 5 days after challenge

Haagmans et al., in preparation
Vaccination of macaques with inactivated SARS CoV: Virus neutralizing antibody titers after 2 vaccinations

Dose and adjuvant dependent VN antibody induction.
Vaccination of macaques with inactivated SARS CoV: SARS CoV PCR titers in the lungs 5 days after challenge

Dose and adjuvant dependent protection!
Eosinophil counts in the bronchus of macaques vaccinated with inactivated SARS-CoV with alum
Presence of IL-13 mRNA in SARS-CoV challenged vaccinated macaques

![Bar charts showing relative transcript numbers for IL-4, IFN-γ, and IL-13 under different conditions: PBS, SARS-CoV, vaccine + SARS-CoV.](chart)
Order Mononegavirales, family Paramyxoviridae

Morbilliviruses: a continuing story!!!
Morbilliviruses crossing species barriers

Antarctic 1955: CDV in Crabeater seals

CDV in Baikal seals
Nature 1988

CDV in Caspian seals
EID 2000

CDV in Serengeti lions
Vaccine 1994

DMV in Med. monk seals
Nature 1997

CDV in Jap. macaques
Vet. Microbiol 1989
Morbilliviruses crossing species barriers

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CDV in Baikal seals
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Vaccine 1994

DMV in Med. monk seals
Nature 1997

CDV in Jap. Macaques
Vet. Microbiol 1989

should we continue measles vaccination for ever?
Evaluation of new generation MV vaccines

Protective Immunity in Macaques Vaccinated with a Modified Vaccinia Virus Ankara-Based Measles Virus Vaccine in the Presence of Passively Acquired Antibodies

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Evaluation of alternative vaccination routes

Measles vaccination of macaques by dry powder inhalation

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Available online 23 October 2006
Evaluation of alternative vaccination routes

Aerosol measles vaccination in macaques: Preclinical studies of immune responses and safety

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Available online 30 June 2006
CONCLUSIONS:

- In the past decade many “new” respiratory viruses have been identified.
- Most of these with novel molecular techniques.
- Several are the result of inter-species transmission.
- The relative clinical impact of most is not yet known.
- Besides influenza vaccines, no respiratory virus vaccines are used.
- Candidate vaccines are being developed against some of these.
- Novel administration routes (e.g. intranasal, dry powder) are explored.
- Safety and efficacy of most of these vaccine candidates is not yet clear.
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- Novel administration routes (e.g. intranasal, dry powder) are explored.
- Safety and efficacy of most of these vaccine candidates is not yet clear.
- Rapid collaborative response may contain emerging respiratory viruses.
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