Imperial College London

Veterinary Vaccinology Network Conference
Birmingham, 17th February 2015

Disease and protective immunity in respiratory viral infections

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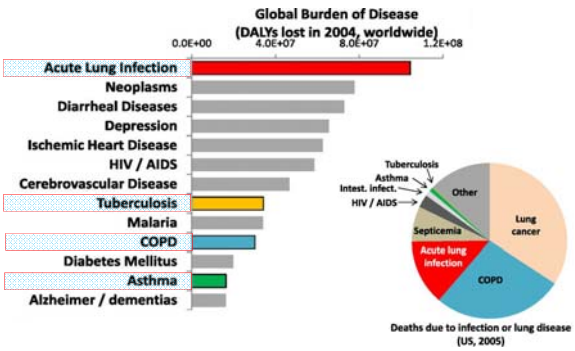
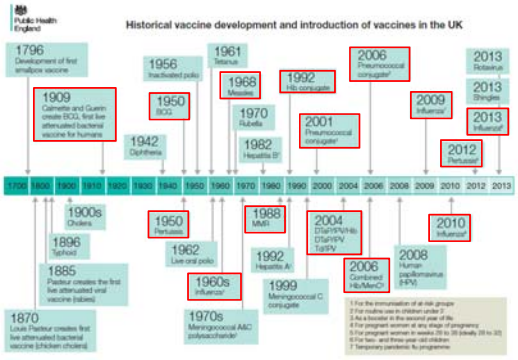
Respiratory Infection and the Impact of Pulmonary Immunity on Lung Health and Disease

Joseph P. Mizgerd[1]

Am J Respir Crit Care Med Vol 186, Iss. 9, pp 824-829, Nov 1, 2012



How are vaccines against respiratory diseases doing?



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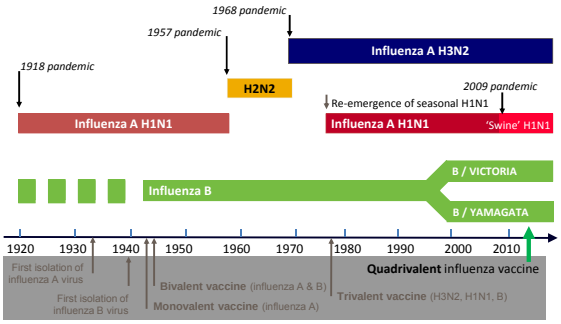
Global circulation of influenza viruses and history of influenza vaccine development



McCullers JA, Huber MC. *Hum Vaccin Immunother* 2012;8:34-44 (adapted).

Increased Risk of Noninfluenza Respiratory Virus Infections Associated With Receipt of Inactivated Influenza Vaccine

Clinical Infectious Diseases 2012;54(12):1778–83

Benjamin J. Cowling,[1] Vicky J. Fang,[1] Hiroshi Nishiura,[1,2] Kwok-Hung Chan,[2] Sophia Ng,[1] Dennis K. M. Ip,[1] Susan S. Chiu,[4] Gabriel M. Leung,[1] and J. S. Malik Peiris[1,5]

We randomized 115 children to trivalent inactivated influenza vaccine (TIV) or placebo. Over the following 9 months, TIV recipients had an increased risk of virologically-confirmed non-influenza infections (relative risk: 4.40; 95% confidence interval: 1.31-14.8). Being protected against influenza, TIV recipients may lack temporary non-specific immunity that protected against other respiratory viruses.

Table 3. Incidence Rates of Respiratory Virus Detection by Reverse-Transcription Polymerase Chain Reaction and Multiplex Assay

	TIV (n = 69)			Placebo (n = 46)			
Variable	No.	Rate[a]	(95% CI)	No.	Rate[a]	(95% CI)	*P* Value
Any seasonal influenza	3	58	(19–180)	3	88	(28–270)	.61
Seasonal influenza A (H1N1)	2	39	(10–160)	2	59	(15–240)	.68
Seasonal influenza A (H3N2)	1	19	(0–140)	0	0	(0–88)	.31
Seasonal influenza B	0	0	(0–58)	1	29	(4–210)	.17
Pandemic influenza A (H1N1)	3	58	(19–180)	0	0	(0–88)	.08
Any noninfluenza virus[b]	20	390	(250–600)	3	88	(28–270)	<.01
Rhinovirus	12	230	(130–410)	2	59	(15–240)	.04
Coxsackie/echovirus	8	160	(78–310)	0	0	(0–88)	<.01
Other respiratory virus[c]	5	97	(40–230)	1	29	(4–210)	.22
ARI episode with specimen collected but no virus detected	19	369	(235–578)	14	412	(244–696)	.75
ARI episode with no specimen collected	41	796	(586–1080)	28	824	(569–1190)	.89

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Current issues in influenza vaccinology

- Do the current vaccines work (enough)?
- How to they work (when they do)?

- Drive towards universal durable protection
- Debates over gain-of-function research
- Cross reactive stalk/stem antibodies

- Novel viral antigens many be weakly antigenic
- Vaccine manufacture needs to speed up in pandemics

Rolf M. Zinkernagel, Nobel Prize Winner for Medicine 1996

Dr. Zinkernagel, which arm of the human immune response has the potential to protect against diseases?

Antibodies. They are the key. The course of any infection depends on the host's response, and I believe that this has to be through antibodies http://www.medscape.com/viewarticle/564375

7

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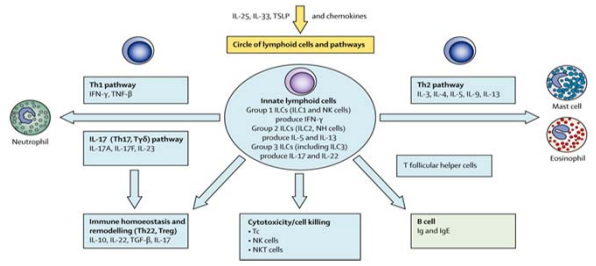
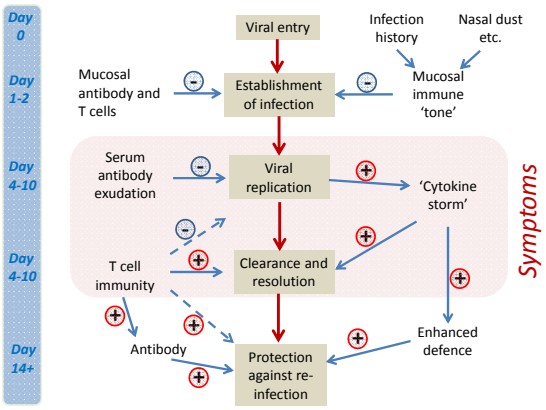
Microbes and mucosal immune responses



Lancet 2013; 381: 861-73
Published Online February 19, 2013
http://dx.doi.org/10.1016/S0140-6736(12)62202-8

Trevor T Hansel, Sebastian L Johnston*, Peter J Openshaw*



9

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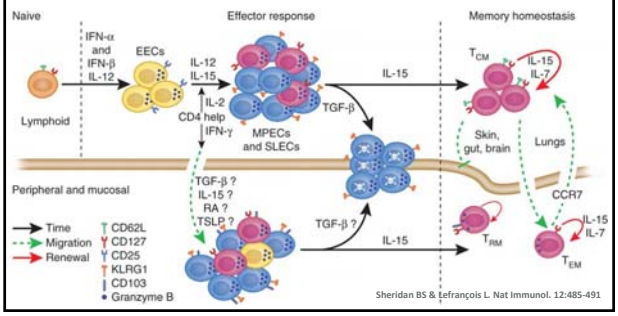
Effector T cells become resident memory T cells (T_{RM}) enabling an early rapid response on reinfection

do not recirculate; maintained by self renewal



Sheridan BS & Lefrançois L. Nat Immunol. 12:485-491

Outline

1. Observational study of pH1N1: MOSAIC

2. Human challenge study of RSV infection

11

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Death from influenza: family clustering

Gustav Klimt 'Death and Life' 1910

Egon Schiele 1890 –1918

Comparative community burden and severity of seasonal and pandemic influenza: results of the Flu Watch cohort study

Lancet Respir Med 2014; 2: 445–54
Published Online March 17, 2014
http://dx.doi.org/10.1016/S2213-2600(14)70034-7

Aim: Intensive community surveillance of burden/severity of seasonal/pandemic influenza to determine degree by which traditional surveillance underestimates flu burden

Method:
Serology, weekly reporting and RT-PCR for flu from nasal swabs
Community surveillance in England 2006–11
5448 person-seasons of follow-up

Findings:
- **~75%** of flu infections are **asymptomatic**
- **4x rise** in strain-specific serology in **18%** of unvaccinated people each winter
- **25% of seroconversions were PCR⁺**
- **17%** of PCR-confirmed flu = medical attendance

pH1N1/09 was less severe than H3N2

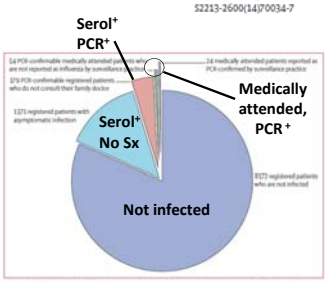

Figure 3: Number of expected events in a surveillance practice serving a population of 10 000 people

13

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Case 1

29 y, white-British man
Well-controlled asthma
10 cigarettes/day
School caretaker
Wife, young daughter
Hx:
24h: Fever, cough, aches
2 h: Breathless at rest

No wheeze; O_2 sat 92%
Raised pulse, breathing fast
pH1N1 RNA detected
• Tamiflu 75 mg bd po 5/7
•IV clarithromycin 500 mg bd

Day 3: well; sent home

Imperial College Healthcare NHS Trust

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Case 2

39 y, Pakistani Taxi driver (UK 12 years)
10 cigarettes/day; Wife + 2 daughters
Hx:
5 d: 'Feverish flu', Dry cough, nausea/anorexia
24 h: Increasing SOB at rest; fast pulse/resps.
O_2 sat 72% on air, 80% on 15L/min oxygen
Tamiflu 75 mg bd po + antibiotics
Day 1
Intubated and ventilated
pH1N1 PCR⁺ day 2 (NP swab/ET aspirate)
Acute renal injury, ARDS
Day 19
Discharged from ITU
Day 23
Allowed home

Imperial College Healthcare NHS Trust

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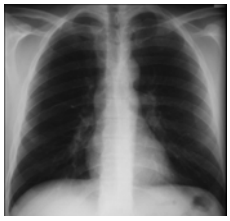
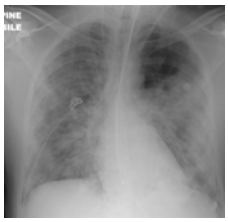
Key unknowns about severe influenza





1. **Why do some have mild disease and others severe?**
2. **What determines the variable outcome?**

16

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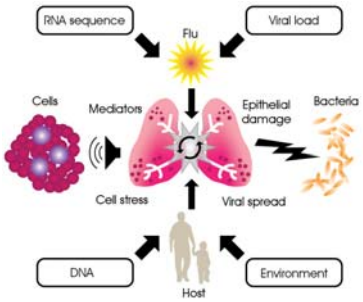
What Causes Severe Disease?



Need to study host, pathogen and co-pathogen

17

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MOSAIC INVESTIGATORS

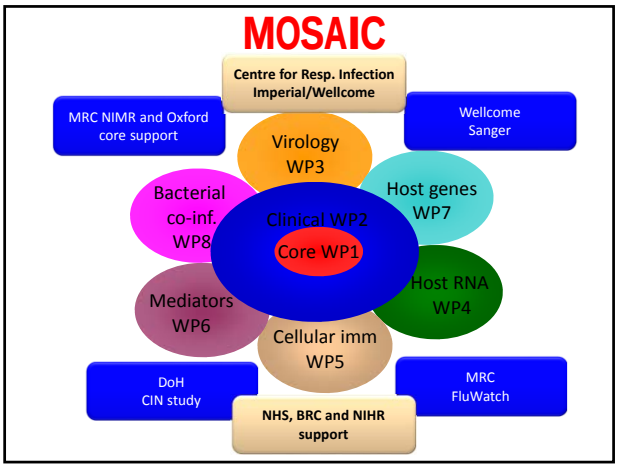
Imperial College London: Deborah Ashby, Paul Aylin, Wendy Barclay, Stephen Brett, William Cookson, Alison Holmes, Tracy Hussell, Trevor Hansel, Onn Min Kon, Michael Levin, Miriam Moffatt, Simon Nadel, Peter Openshaw
Liverpool: Paul McNamara, Calum Semple, Peter Simmonds, Rosalind Smyth, Stephen Gordon
Nottingham: Jonathan Van Tam
Oxford: Ling-Pei Ho, Andrew McMichael
Edinburgh: Kenneth Baillie, David Hume, Tony Nash
Glasgow: William Carman, Walt Adamson
HPA: Maria Zambon
NIMR: Anne O'Garra
UCL/Sanger: Paul Kellam, Andrew Hayward
The patients and their relatives

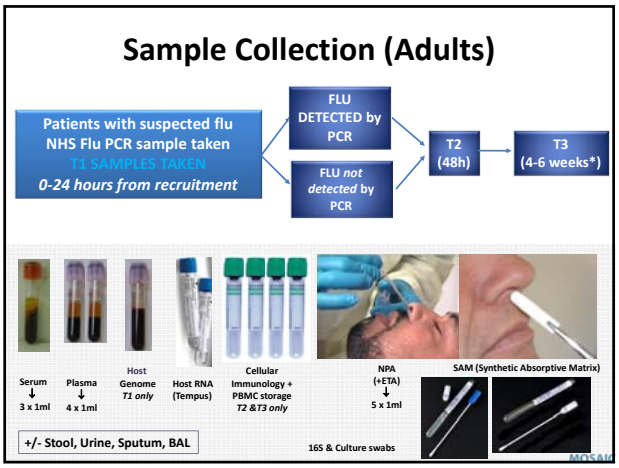
Jake Dunning

MOSAIC support
Mary Cross
Lindsey Anne Cumming
Matthew Minns
Tom Ford

Mark Walport
John Saville
Sally Davies
Gordon Duff

MRC Medical Research Council
wellcome trust sanger institute





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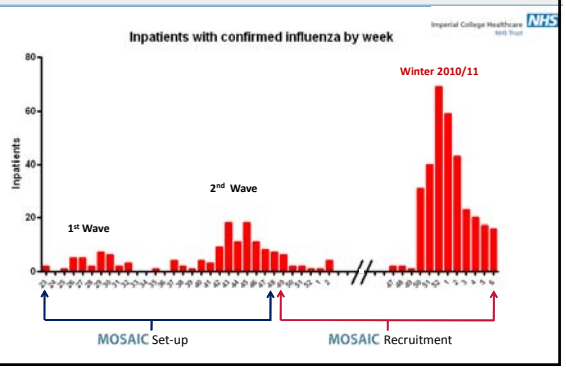
MOSAIC timing



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MOSAIC

255 patients with influenza-like illness
172 (65%) with PCR-confirmed influenza

45 page booklet (Flu-CIN) | Extensive clinical information | Samples from multiple timepoints | Respiratory, blood and other samples | 8000 sample biobank

Virology and genomics | Molecular bacteriology | Mediators and cellular immunology | Transcriptomics | Host genomics

+/- community (mild) controls, matched healthy controls, ILI controls

Probably the world's most comprehensive study of the causes of severe pandemic influenza

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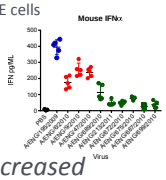
Human adaptation of H1N1 during 3 successive waves

- Weight loss in mice varied between viral isolates
- 3rd wave virus induced less IFN-α in the infected mouse lungs
- 2nd and 3rd wave viruses replicated more rapidly in huAE cells

Mutations:

- accumulated over time (including in NS1)
- HA/NA mutations increased binding to α-2,6 sialic acid
- PA (N321K) mutation enhanced polymerase activity

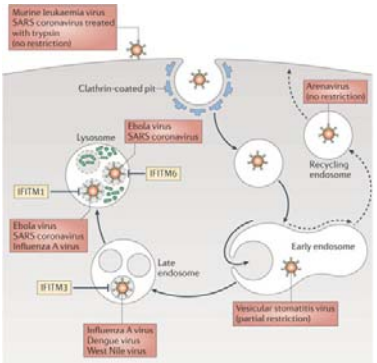


Multiple mutations contribute to increased fitness in mouse/human cells

Ruth A. Elderfield., Simon J. Watson., Alexandra Godlee., Walt E.Adamson., Catherine I. Thompson., Jake Dunning., Mirian Fernandez-Alonso., Deena Blumenkrantz., Tracy Hussell ., The MOSAIC investigators, Maria Zambon., Peter Openshaw., Paul Kellam.and Wendy S. Barclay..

Published 10 September 2014, doi: 10.1128/JVI.01636-14

IFITMs in viral infection



IFN-induced transmembrane protein (IFITM)-mediated restriction activity correlates with the site of viral fusion

Arenaviruses follow the recycling pathway of their common receptor, transferrin receptor 1, and are not subject to IFITM-mediated restriction

Influenza A viruses fuse in late endosomes and are restricted by IFITM proteins, particularly by IFITM3

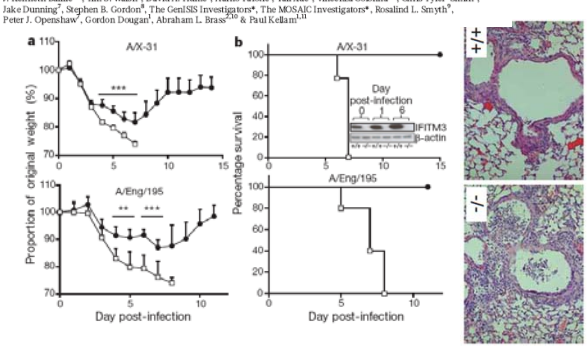
Diamond MS and Farzan M. Nat Rev Immunol. 13:46-57

24

IFITM3 restricts the morbidity and mortality associated with influenza

Nature nature10921.3d

Aaron R. Everitt[1], Simon Clare[1], Thomas Pertel[2], Sinu P. John[2], Rachael S. Wash[1], Sarah E. Smith[1], Christopher R. Chin[2], Eric M. Feeley[2], Jennifer S. Sims[2], David J. Adams[3], Helen M. Wise[3], Leanne Kane[1], David Goulding[1], Paul Digard[3], Verneri Antrila[1], J. Kenneth Baillie[4,5], Tim S. Walsh[5], David A. Hume[4], Aarno Palotie[1], Yali Xue[1], Vincenza Colonna[1,6], Chris Tyler-Smith[1], Jake Dunning[7], Stephen B. Gordon[8], The GenISIS Investigators*, The MOSAIC Investigators*, Rosalind L. Smyth[9], Peter J. Openshaw[7], Gordon Dougan[1], Abraham L. Brass[2,10] & Paul Kellam[1,11]



IFITM3 restricts the morbidity and mortality associated with influenza

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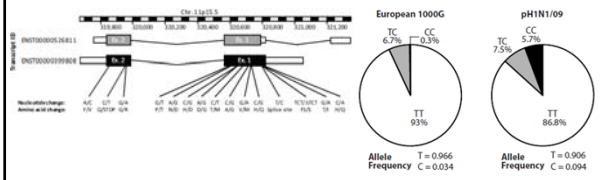
IFITM3 genes in MOSAIC cases of severe influenza



- Screened 53 hospitalised cases of severe influenza
- Sequenced 1.8kb of IFITM3 locus, including exons, intron and untranslated
- IFITM3 splice variant with 21 aa N terminal deletion identified
- Differ significantly from matched Europeans (WTCCC1: p<0.00007; Fisher's exact test)
- Genotypes differ from Hardy Weinberg equilibrium (p=0.003)

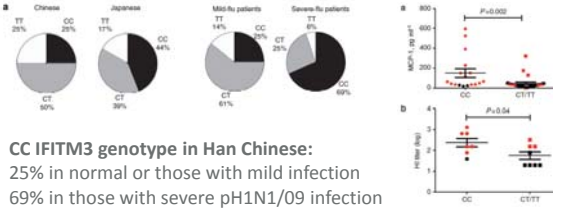
26

Interferon-induced transmembrane protein-3 genetic variant rs12252-C is associated with severe influenza in Chinese individuals

nature COMMUNICATIONS

Yang-Hong Zhang[1*], Yan Zhao[1*], Ning Li[2*], Yan-Chun Peng[3], Eleni Giannoulatou[3], Rong-Hua Jin[1], Hui-Ping Yan[1], Hao Wu[1], Jin-Hua Liu[1], Ning Liu[1], Da-Yan Wang[4], Yue-Long Shu[4], Ling-Pei Ho[3,5], Paul Kellam[6,7], Andrew McMichael[3] & Tao Dong[3,5]

Normal population **infected Chinese** **MCP1 and Viral load**



CC IFITM3 genotype in Han Chinese:
25% in normal or those with mild infection
69% in those with severe pH1N1/09 infection

CC genotype: 6x greater risk for severe infection than CT/TT

Population-attributable risk of 54.3% for severe infection in the Chinese population studied compared with 5.4% in Northern Europeans.

27

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Soluble mediators in severe influenza

Generally:
1. Many show upward trend with severity
2. Some show gradients towards mucosa

But:
1. Some decline with severity
2. Some are more abundant in serum than mucosa

28

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What have we learnt from MOSAIC?

Problems with studying outbreaks:
1. Almost impossible from a standing start
2. We need to improve if we want to do this again

The integrated host/pathogen approach:
1. Needs great collaborators
2. Need excellent healthcare infrastructure
3. Is a very long game…

Dans ses écrits, un sàge Italien
Dit que le mieux est l'ennemi du bien.

In his writings, a wise Italian…
says that the best is the enemy of the good
Voltaire *La Bégueule*

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Influenza vaccines

- Current vaccines are not good enough
- They do not fully recapitulate natural immunity

- We need universal flu vaccines
- Work on cross-reactive stalk/stem antibodies
- Need long duration of protection

- Mucosal immunity and needle free vaccines ideal
- Need to speed up production in pandemics

Outline

1. Observational study of pH1N1: MOSAIC

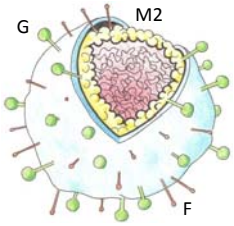
2. Human challenge study of RSV infection

31

The discovery and naming of RSV

Morris, Blount, & Savage Proc. Soc. Exp. Biol. Med. 1956:
Coryza in zoo chimps with coryza; recovered virus and infected naïve chimps.
Named it the Chimpanzee Coryza Agent (CCA)

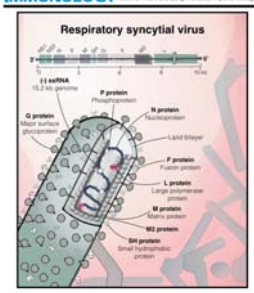
The Savage agent



32

Morris, Blount, & Savage Proc. Soc. Exp. Biol. Med. 1956:
Named the Chimpanzee Coryza Agent (CCA)

frontiers in IMMUNOLOGY
Laura Lambert, Agnes M. Sagfors, Peter J. M. Openshaw *† and Fiona J. Culley *†
National Heart and Lung Institute, Imperial College London, London, UK
REVIEW ARTICLE
Published: 29 September 2014
10.3389/fimmu.2014.00466



- A member of the *Paramyxoviridae* family
- Single stranded, negative sense RNA virus
- ~ 15,200 nucleotides
- Transcribed into 11 subgenomic mRNAs

The Savage agent

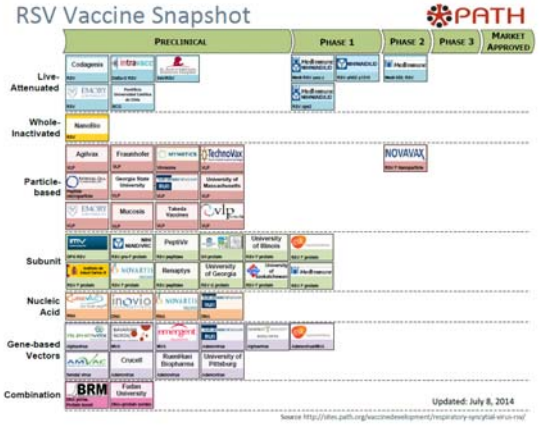
Respiratory Syncytial Virus

- World-wide distribution, winter epidemics
- Infects 65% of children in first year of life
- Two serogroups, but reinfects with ease

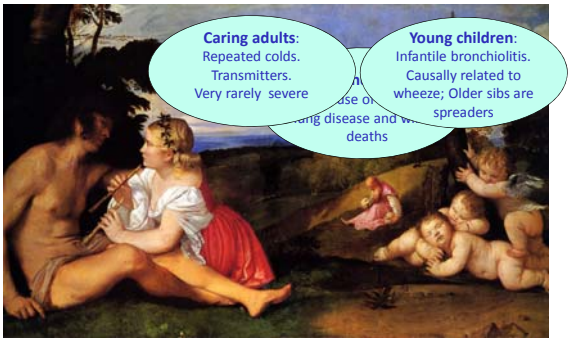
Causes:

- Hospital admission in 2-3% of infants
- 70% of bronchiolitis in infancy
- Coughs and colds in adults
- Wheezy RTI in kids <5 years, asthmatics *etc*

34



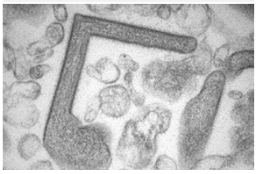
Respiratory syncytial virus through the ages



Three ages of man, Titian, National Gallery of Scotland

Immunological facts and puzzles

RSV



- Antibody gives only partial protection
- Patients with T cell immune defects have prolonged infection
- Formalin inactivated vaccine made disease worse
- Ex-bronchiolitics often wheeze and/or have 'asthma'

37

Human challenge study design

- **Healthy**, aged 18 – 55 years
- Intranasal 10^4 pfu RSV A **Memphis 37**
- Keep in seclusion from D-1 to D10
- Intensive daily sampling

- Follow-up:
  - day 14 (airway)
  - day 28 (airway and blood)

Dr Max Habibi

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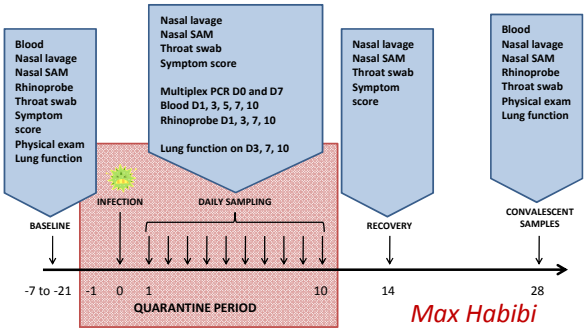
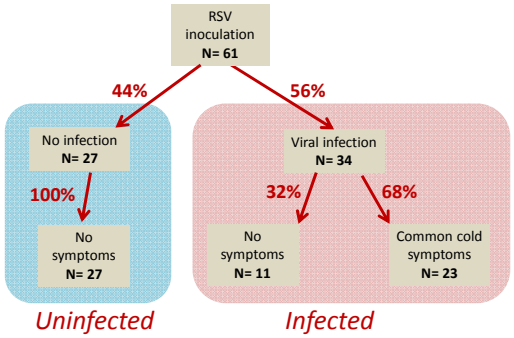
Adult volunteer RSV infection protocol



Max Habibi and Chris Chiu

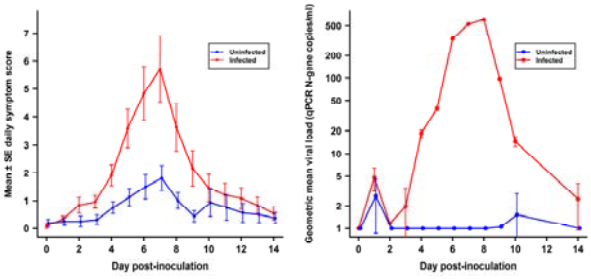
Infection rates and colds (n=61)



No difference between males and females
No relationship between age and infection rate or colds

40

Symptoms and RSV load



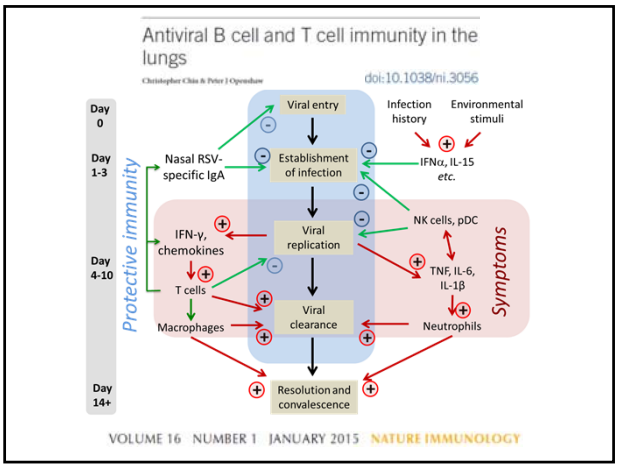
Questions

- Why do some subjects ... ted?
- Why ... cted people not develop

It's not all down to antibody

What explains this variability?

What about mediator release?



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RSV clinical study team

Chris Chiu and Max Habibi

Trevor T Hansel
Agnieszka Jozwik
Allan B Paras
Aleks Guvenel
Spiros Makris
David Jackson

Collaborators and advisors:

John De Vincenzo, Tom Wilkinson,
Rob Lambkin-Williams, Sebastian L Johnston

44