GEIG
Groupe d'Expertise et d'Information sur la Grippe

23e Rencontres du GEIG
Retours d'expériences sur la pandémie H1N1
jeudi 25 et vendredi 26 novembre 2010

Statut immunitaire et réponses post-vaccination H1N1 pandemique

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"Vaccination strategies and immune memory"

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Bilan des questions sur l'immunité contre le virus H1N1 California 2009

Avant/ la pandémie	Données littératures
1. Quel est l'état de l'immunité contre le virus H1N1 California 2009?	
○ Le titre HAI (Inhibition de l'hémagglutination)	Dépendant de l'âge des sujets
○ L'immunité cellulaire T CD4 et CD8	Hétérogène
2. La vaccination saisonnière protège-t-elle contre le virus H1N1 California 2009?	Non *Cross-protection…?*
3. Quelle est l'efficacité des réponses vaccinales après vaccination contre le virus pandémique chez les sujets adultes?	80-100% (HAI ≥40) and >4x
4. Induit-on une réponse cellulaire après vaccination contre le virus H1N1?	CD4 oui CD8 (en théorie)?

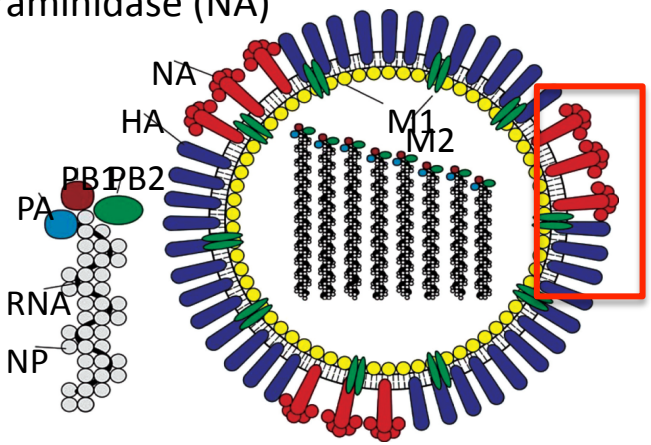
Après la pandémie	
4. Quel est l'état de la mémoire immunitaire après vaccination?	inconnu
5. Est-elle comparable à la l'immunité après infection « contrôlée » ou infection « formes graves »?	inconnu

Baseline immunity (HAI titers) to Pandemic H1N1

Publication	Periode of inclusion * Serum samples	Age of subjects	% subject HAI titers ≥40 Baseline H1N1pdm vaccination
Hancok et al NEJM Sep 2009	*** 1971 2002-feb 2009**	**< 30 yrs (n=107) > 60 yrs (=115)**	**4% 34%**
Zhu et al NEJM Oct 2009	Spring 2009	18-60 yrs ≥60 yrs	4.3% (2.8-6.3%) 4% (2.5-6.0%)
Zimmer et al Plos One July 2010	• 2008 • Post- First wave	Adult 19- 60 yrs	6% 14-22%
Greenberg et al NEJM Dec 2009	**Begining of the peak**	**18-64 yrs**	**27-32%**
Labrosse et al. Plos One 2010	Aug 2009-Oct 2009	unknown	1/10 (HAI) 9/10 (N titers VLP)
Kung et al Vaccine 2010	Area of low epidemic level of panemic	<60 yrs (n=186)	4.8%
Madhum et al Vaccine 2010	**Oct 2009- March 2010**	**21-67 yrs Health care**	**13%**

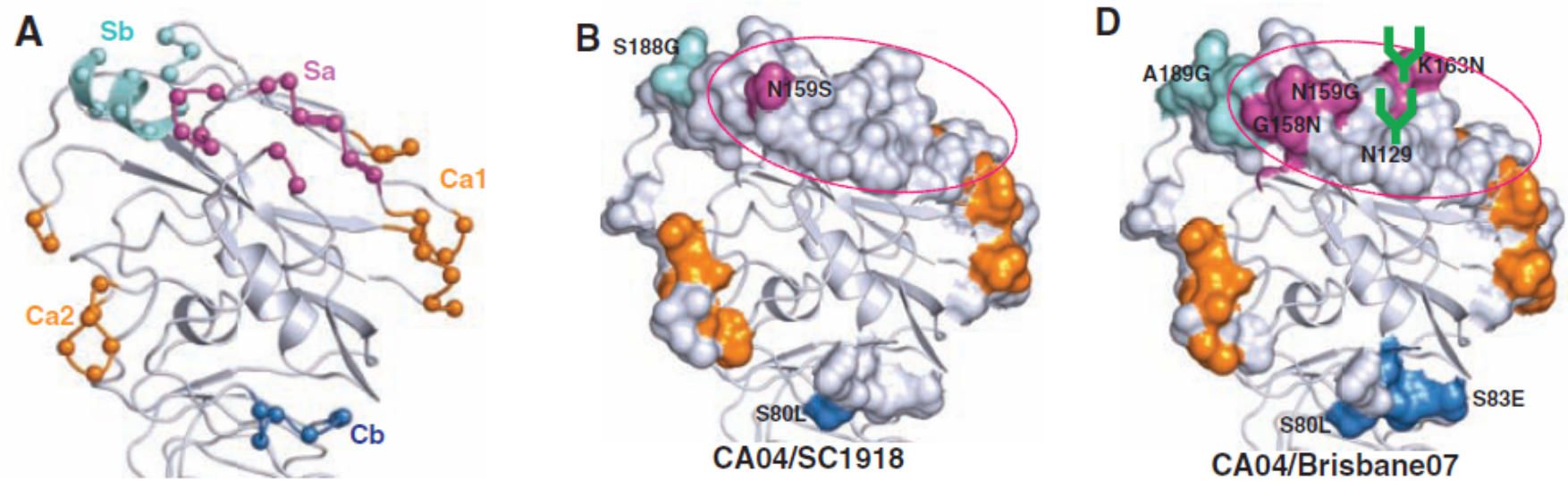
Anti-influenza humoral immune responses

Hemagglutinine (HA)
neuraminidase (NA)

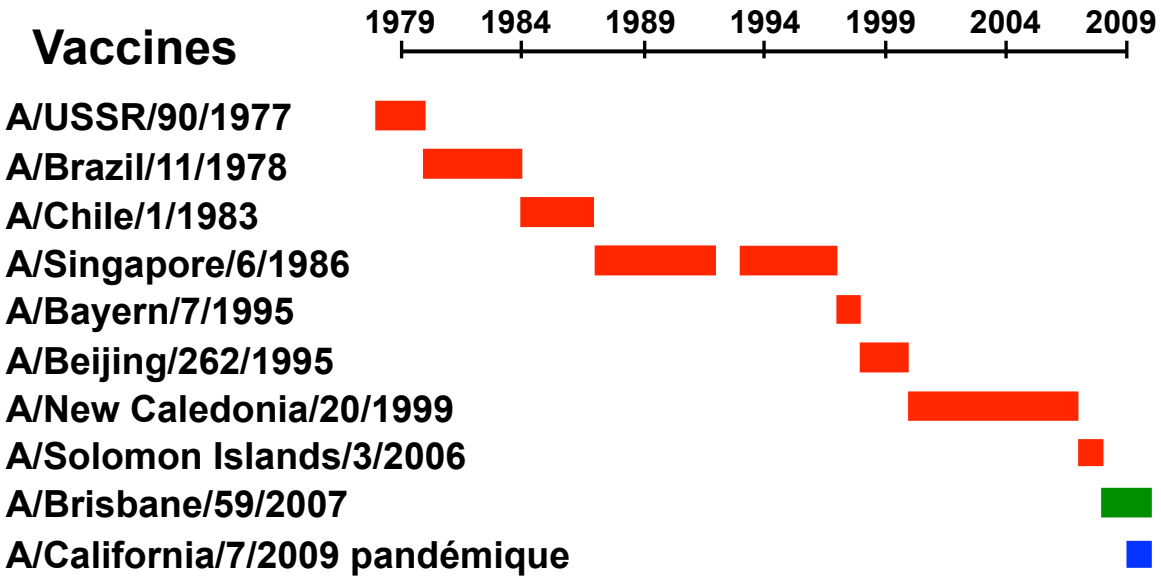


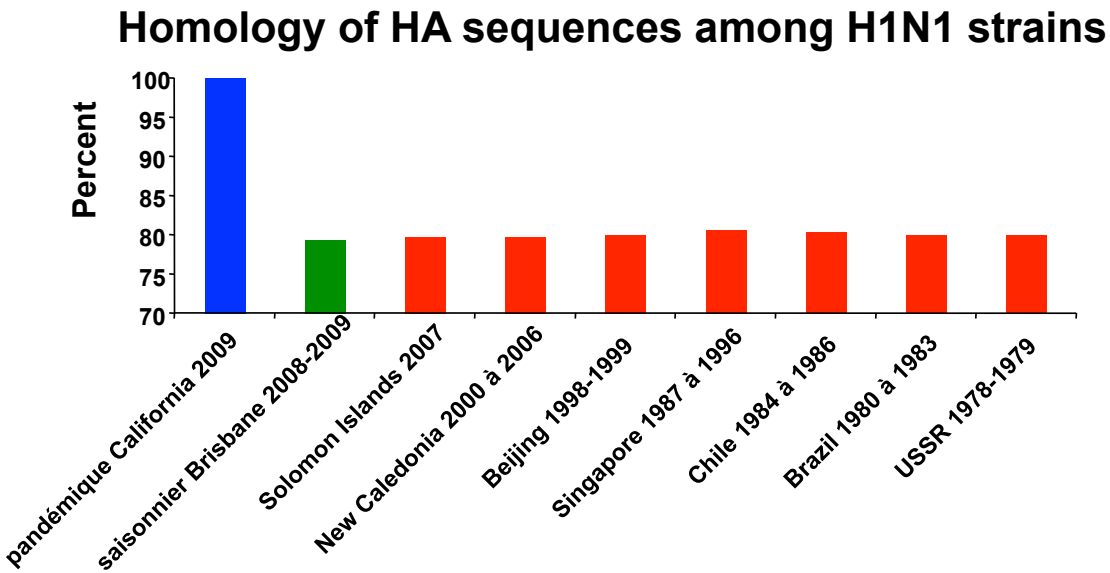
Antigenic sites	No. amino acids involved	No. of amino acids identical to SC1918	
		BR2007	CA2009
Sa	13	8	12
Sb	12	4	10
Ca	19	13	13
Cb	6	2	5

doi:10.1371/journal.pone.0008553.t001



Divergence of antigenic site of influenza viruses





Immune responses to influenza viruses

CD4 T cells

IFNg, TNFa, IL-2, Chemokines, CD40L

Cytokine release
T help
Switch isotypique

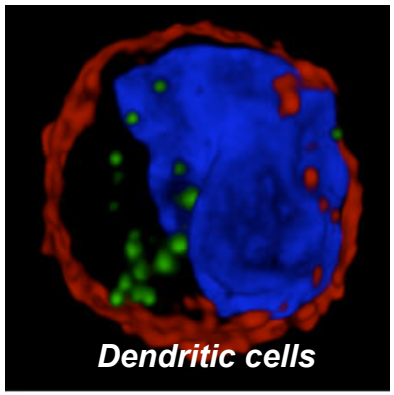
Virus elimination
Control of the viral infection

Neutralization
Inhibition
Phagocytosis
ADCC

B cells

Systemic response
(IgG, IgA)

Mucosal response
(IgG IgA)



from A Smed Sörensen
MHC class II (red) virus (green)

CD8 cytotoxic T cells

Killing of infected cells

Effector CD8 T cells

IFNg, TNFa, IL-2, Chemokines, CD40L

NK cells

- **IgA: first line of defense in respiratory tract (prevent viral infection)**
  Renegar et al 2004
- **Mucosal T cell response**
  Arulanandam et al 2001

Diversity of T cell epitopes

Seasonal Virus A/Brisbane/59/2007(H1N1),
Pandemic Virus A/California/04/2009(H1N1)

RED AA changes H1N1 brisbane and california

HLA-I

MKVKLLVLLC TFTATYADTI CIGYHANNST DTVDTVLEKN VTVTHSVNLL ENSHNGKLCL LKGIAPLQLG NCSVAGWILG NPECELLISK 090
MKAILVVLLY TFATANADTL CIGYHANNST DTVDTVLEKN VTVTHSVNLL EDKHNGKLCK LRGVAPLHLG KCNIAGWILG NPECESLSTA 090

HLA-II **HLA-I** **HLA-II**

ESWSYIVEKP NPENGTCYPG HFADYEELRE QLSSVSSFER FEIFPKESSW PNHTVT-GVS ASCSHNGESS FYRNLLWLTG KNGLYPNLSK 180
SSWSYIVETP SSDNGTCYPG DFIDYEELRE QLSSVSSFER FEIFPKTSSW PNHDSNKGVT AACPHAGAKS FYKNLIWLVK KGNSYPKLSK 180

HLA-II **HLA-I** **HLA-II**

SYANNKEKEV LVLWGVHHPP NIGIQKALYH TENAYVSVVS SHYSRKFTPE IAKRPKVRDQ EGRINYYWTL LEPGDTIIFE ANGNLIAPRY 270
SYINDKGKEV LVLWGIHHP TSADQQSLYQ NADTYVFVGS SRYSKKFKPE IAIRPKVRDQ EGRMNYYWTL VEPGDKITFE ATGNLVVPRY 270

HLA-II **HLA-II** **HLA-I**

AFALSRGFGS GIINSNAPMD KCDAKCQTPQ GAINSSLPFQ NVHPVTIGEC PKYVRSAKLR MVTGLRNIPS IQSRGLFGAI AGFIEGGWTG 360
AFAMERNAGS GIIISDTPVH DCNTTCQTPK GAINTSLPFQ NIHPITIGKC PKYVKSTKLR LATGLRNIPS IQSRGLFGAI AGFIEGGWTG 360

HLA-II **HLA-II**

MVDGWYGYHH QNEQGSGYAA DQKSTQNAIN GITNKVNSVI EKMNTQFTAV GKEFNKLERR MENLNKKVDD GFIDIWTYNA ELLVLLENER 450
MVDGWYGYHH QNEQGSGYAA DLKSTQNAID EITNKVNSVI EKMNTQFTAV GKEFNHLEKR IENLNKKVDD GFLDIWTYNA ELLVLLENER 450

HLA-A11

TLDFHDSNVK NLYEKVKSQL KNNAKEIGNG CFEFYHKCND ECMESVKNGT YDYPKYSEES KLNREKIDGV KLESMGVYQI LAIYSTVASS 540
TLDYHDSNVK NLYEKVRSQL KNNAKEIGNG CFEFYHKCDN TCMESVKNGT YDYPKYSEEA KLNREEIDGV KLESTRIYQI LAIYSTVASS 540

HLA-I

LVLLVSLGAI SFWMCSNGSL QCRICI 566
LVLVVSLGAI SFWMCSNGSL QCRICI 566

Cross-reactivity of memory T cells

Table 2. Distribution of epitopes among the influenza proteins

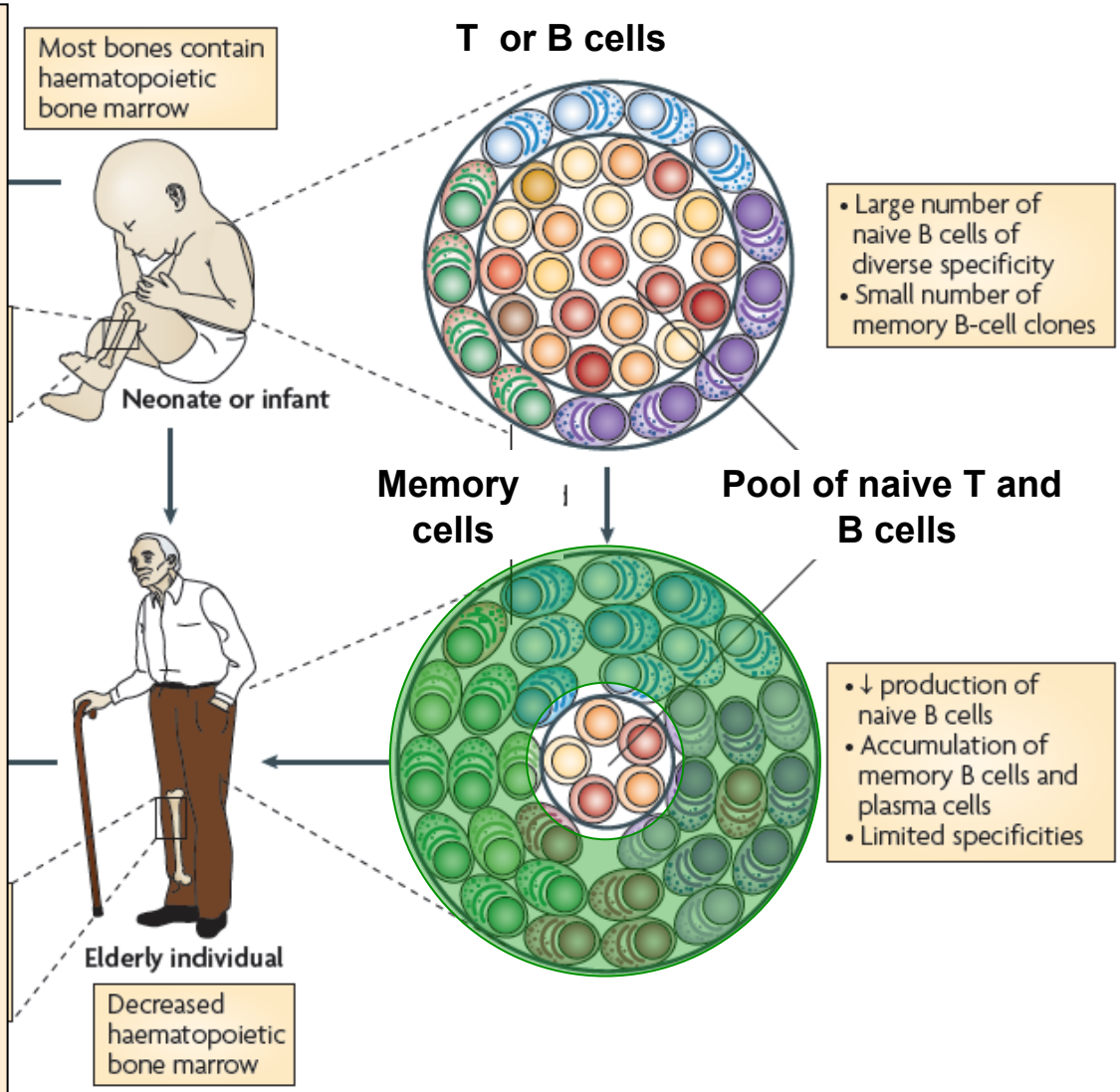
	B-cell		T-cell, CD8+		T-cell, CD4+		Overall		
Protein	Total	Cons.	Total	Cons.	Total	Cons.	Total	Cons.	Cons. (%)
HA	5	1	4	1	34	3	43	5	12
NA	1	0	2	1	3	0	6	1	17
M1	4	1	17	13	28	14	49	28	57
M2	4	1	1	0	3	0	8	1	13
NS1	1	0	2	1	2	1	5	2	40
NS2	0	0	1	1	1	0	2	1	50
NP	9	4	19	15	43	21	71	40	56
PA	0	0	7	4	1	1	8	5	63
PB1	2	1	23	17	21	16	46	34	74
PB1-F2	0	0	0	0	0	0	0	NA	NA
PB2	0	0	2	1	3	1	5	2	40

The total number of epitopes in the H1N1 seasonal flu strains from 1988–2008 (Total) as well as the number of epitopes conserved in swine-origin H1N1 influenza virus (S-OIV) (Cons.) are listed.

Greenbaum et al. PNAS 2009

Limits of the unlimited immune system

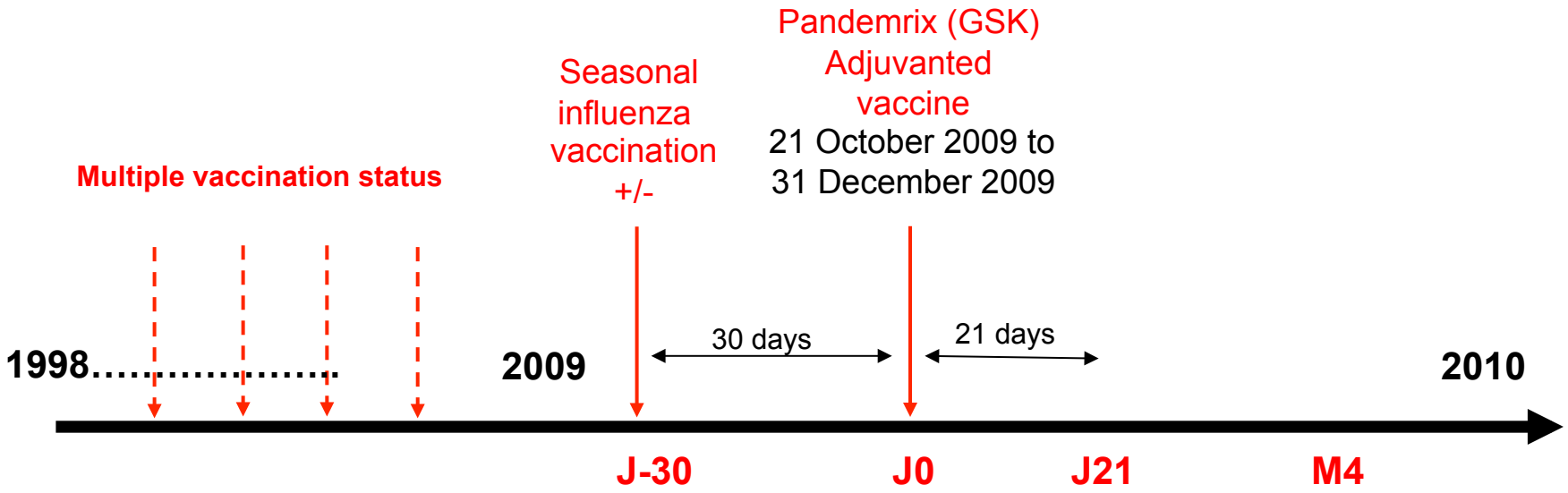
- Target groups:
  - Without immunological experience (or little)
  - **With immunological experience (multiple infection/ multiple vaccination)**
  - With long-term immunological experience **<u>but aged immune system</u>**



Nature Reviews | Immunology

Vaccination against pandemic H1N1 in multiple vaccinated subjects

FLUHOP study group : S Jauréguiberry, F Carrat, O Launay, S Van der Werf et al. (IMMI, INSERM, France)



Hospital staff

Pitié-salpétrière, service des maladies infectieuses
Pitié-Salpétrière, Medecine du Travail
Cochin Saint Vincent de Paul, CIC Vaccinologie

N=147 (drop off = 8) :47ml peripheral blood/ time point

Primary end point:
Inhibition of Hemagglutination Ab (IHA) titers

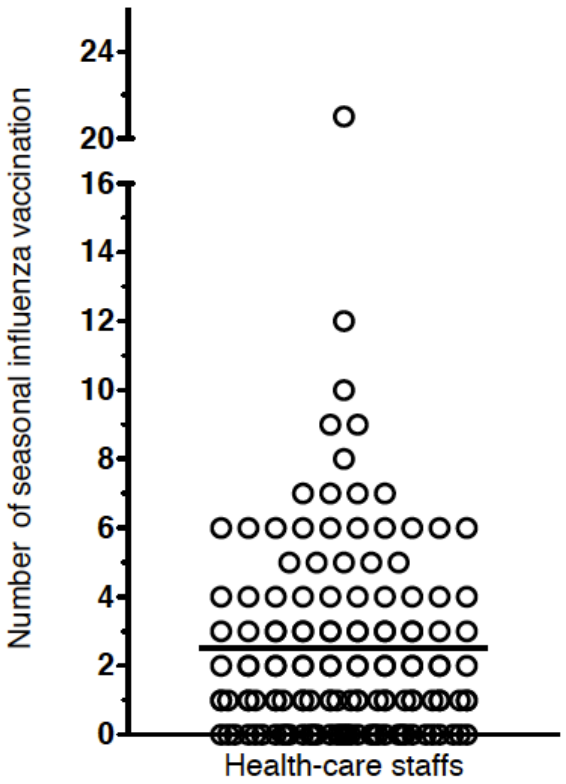
Secondary end points:
B cell responses
T cell responses
Cross-reactivity

Vaccination against pandemic H1N1 in multiple vaccinated subjects

FLUHOP study group : S Jauréguiberry, F Carrat, O Launey, S Van der Werf et al. (IMMI, INSERM, France)

Characteristics	
Age-yr	n=139
Mean±sd	42.69 ±12.42
Median (IIQ)	42.61 (33.39 - 53.44)
Sexe (N (%)	n=139
Male	45 (32.37%)
Female	94 (67.63%)
Occupation	n=128
Medical staffs	84 (65.72%)
Administration-Research staffs	44 (34.38%)
Total number of previous seasonal influenza vaccination	N=127
Mean ± sd	2.59 ± 3.00
Median (IIQ)	2.00 (0.00 - 4.00)
0 seasonal influenza vaccination	32 (25.20%)
1 seasonal influenza vaccination	28 (22.05%)
2 seasonal influenza vaccinations	17 (13.39%)
≥3 seasonal influenza vaccinations	50 (39.37%)
Seasonal BR07 vaccination*	n=127
Yes	84 (66.14%)
No	43 (33.86%)

* BR07: influenza A/Brisbane/59/07(H1N1) (season 2008-2009 and 2009-2010)
All subjects received one dose Pandemrix® vaccines



S Jauréguiberry et al submitted 2010

High level of baseline pandemic H1N1–specific Antibodies (IHA) in multiple vaccinated subjects

Prior H1N1pdm vaccination (Baseline)	**N=139**
GMT (IC_{95}%)	21.40 (17.74-25.81)
Protection titers ≥1:40 - n (%)	**53 (38.13%)**
Protection titers ≥1:80 - n (%)	**26 (18.71%)**
After H1N1pdm vaccination (Day 21)	N=139
GMT (IC_{95}%)	247.07 (208.37-292.97)
Protection titers ≥1:40 - n (%)	138 (99.28%)
Protection titers ≥1:80 - n (%)	128 (92.09%)
Fold increase in GMT titers	11.55 ± 4

GMT: geometric mean titers
H1N1pdm : A/Califonia/7/2009(H1N1)

S Jauréguiberry et al submitted 2010

Pandemic and seasonal H1N1 influenza hemagglutinin-specific T cell responses elicited by seasonal influenza vaccination

Ramu A. Subbramanian[a,*], Saleem Basha[a], Mohamed T. Shata[b], Rebecca C. Brady[a], David I. Bernstein[a]

[a] Cincinnati Children's Hospital Medical Center, Division of Infectious Diseases, Department of Pediatrics, Cincinnati, OH, USA
[b] University of Cincinnati College of Medicine, Division of Digestive Diseases, Department of Internal Medicine, Cincinnati, OH, USA

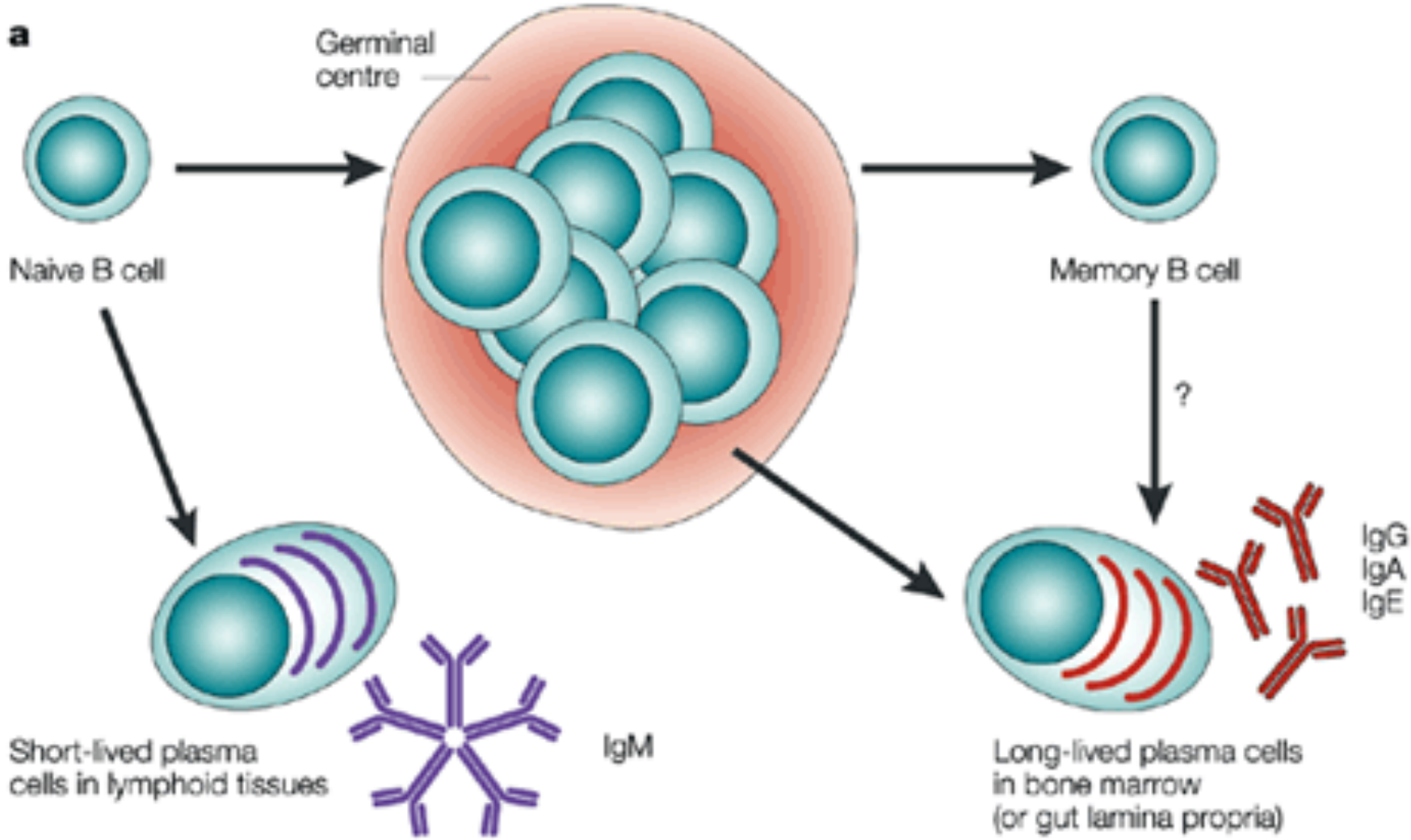
Vaccine 2010 in press

Pandemic HAI titers after recent seasonal vaccination

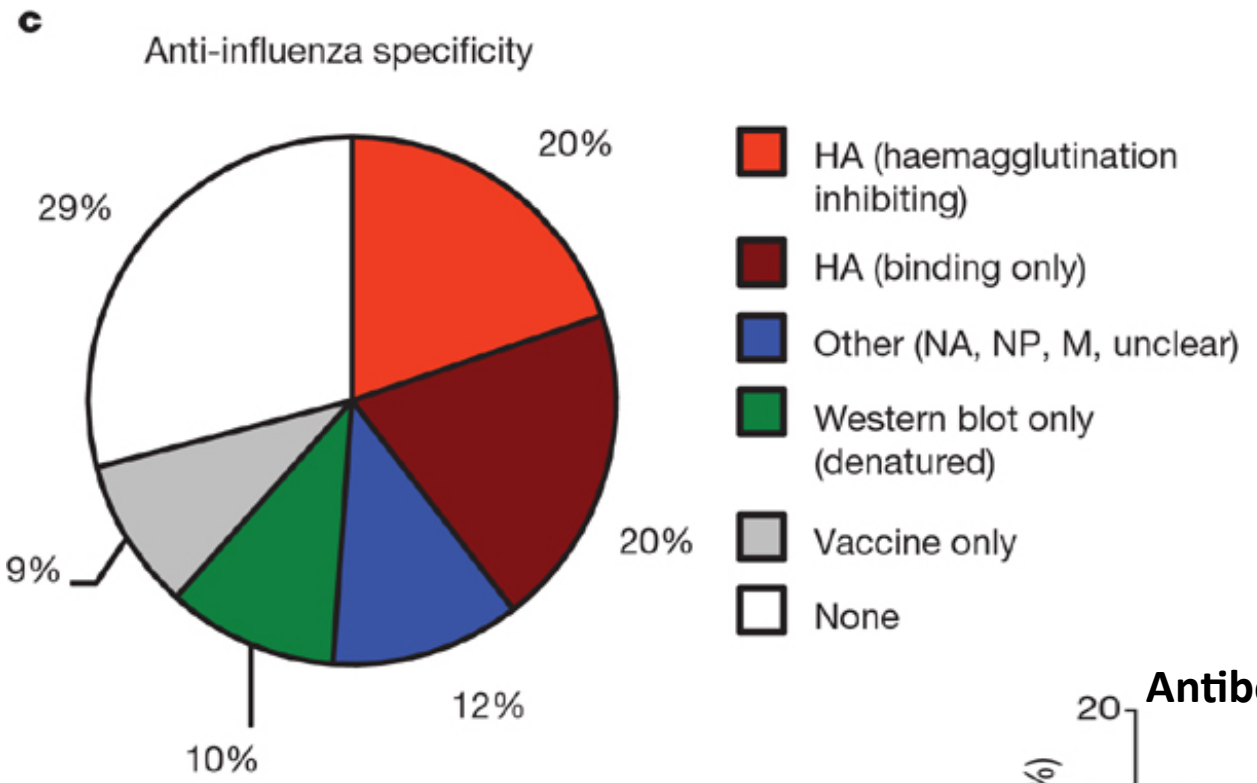
Table 3
Pandemic H1N1 HAI titers elicited by TIV and LAIV in healthy subjects.

HAI titer	All subjects (n = 30)	
	Pre-vaccination	Post-vaccination
GMT (range)	2.4 (2.0–8.0)	2.9 (2.0–6.0)

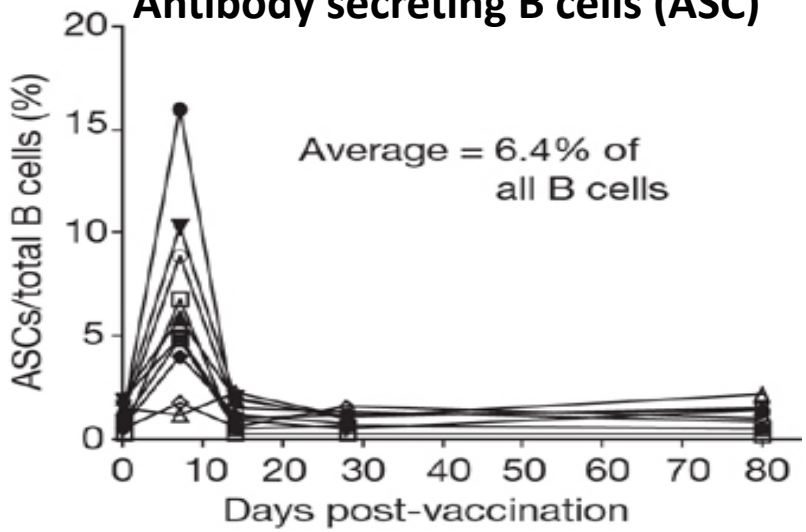
B cell differentiation



Antigenic specificity and B cellular immune responses



Antibody secreting B cells (ASC)



CONCLUSION I : Pandemic H1N1-specific IH Antibodies

✓Baseline (Day 0) HIA titers were surprisingly **≥40 in 38.7%** of subjects.

✓**Significant correlation between high baseline anti-H1N1pdm IHA titers** and **multiplicity of the vaccination status of subjects,** but not occupational work nor age.

✓Subjects vaccinated against **A/Brisbane/59/2007(H1N1) (2008-2009 and/or 2009-2010)** had the highest anti-H1N1pdm antibody titers at baseline.

✓After H1N1pdm vaccination, the GMT increased from 21.4 (Day 0) to 247.07 (Day 21) with protective titers reached in 99.28% of individuals.

✓Subjects **never vaccinated against A/Brisbane/59/2007(H1N1)** and who received H1N1pdm vaccine, **significantly amplified their anti-A/Brisbane/59/2007(H1N1) antibody titers**.

Recent publication :
H1N1 Pandemrix vaccination in Norwegian health care workers (Vaccine 2010)

Baseline IHA titers ≥40: 13% (periode of inclusion Oct 2009- March 2010)
Only 3% received seasonal influenza vaccine

B cell responses and cross-reactivity

- Highly neutralizing antibodies directed against conserved HA epitopes

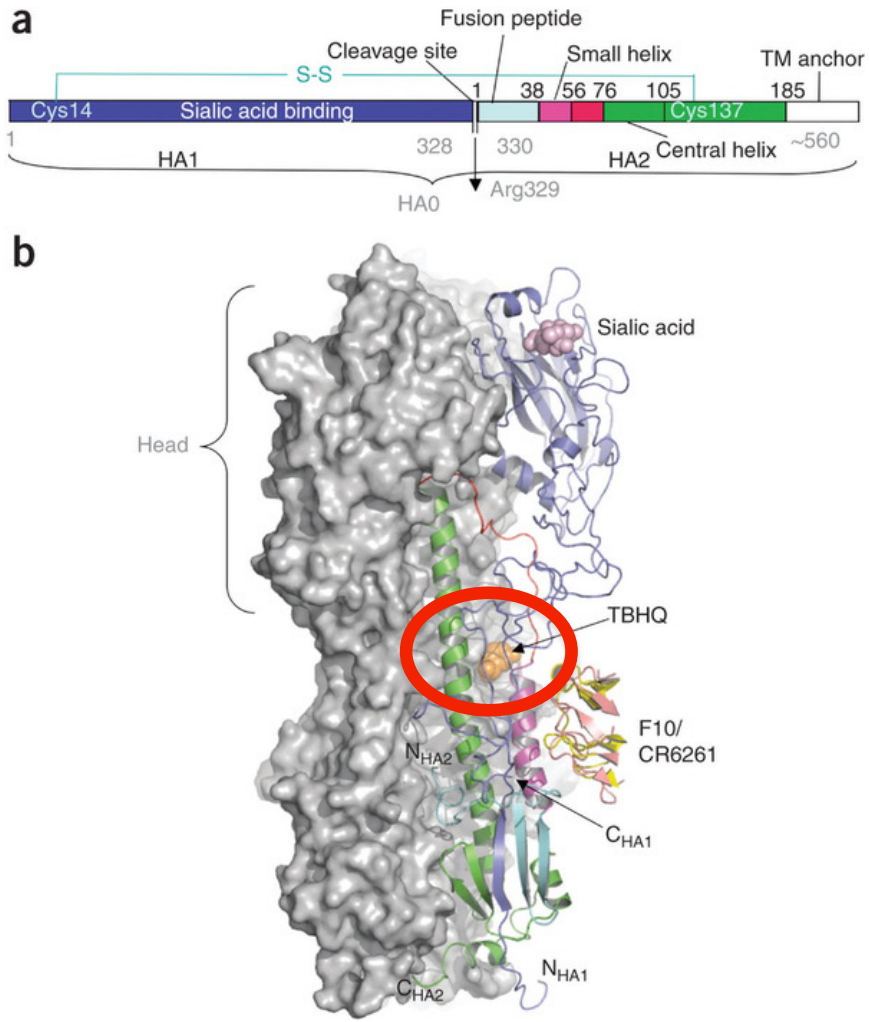
Das et. al, nature 2010

- Seasonal vaccination induces the development of polyclonal heterosubtypic neutralizing antibodies that cross react with HA of different subtypes (H1-H3-H5)

Corti et. al, JCI 2009

- Mice model of protective cross-reactive antibodies against H1N1pdm

Boon et al. JVI 2010



Protective Efficacy of Seasonal Influenza Vaccination against Seasonal and Pandemic Influenza Virus Infection during 2009 in Hong Kong

Benjamin J. Cowling,[1] Sophia Ng,[1] Edward S. K. Ma,[2] Calvin K. Y. Cheng,[1] Winnie Wai,[1] Vicky J. Fang,[1] Kwok-Hung Chan,[2] Dennis K. M. Ip,[1] Susan S. Chiu,[3] J. S. Malik Peiris,[2,4,a] and Gabriel M. Leung[1,a]

[1]Infectious Disease Epidemiology Group, School of Public Health, Li Ka Shing Faculty of Medicine, Departments of [2]Microbiology and [3]Pediatrics and Adolescent Medicine, The University of Hong Kong, and [4]HKU-Pasteur Research Centre, Hong Kong Special Administrative Region, China

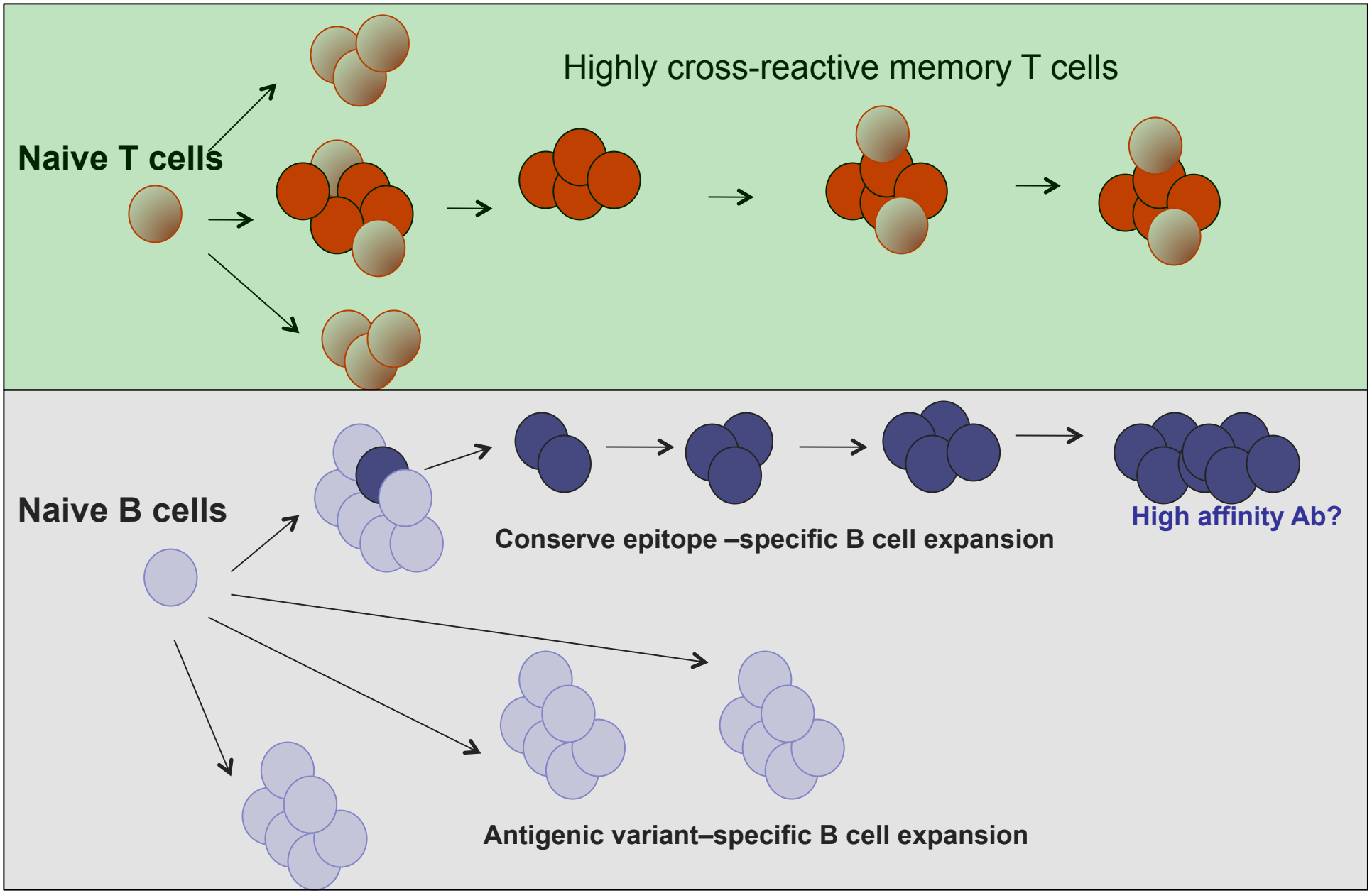
CID 2010:51 (15 December) • Cowling et al

Background. The relationship between seasonal influenza vaccine and susceptibility to 2009 pandemic A/H1N1 virus infection is not fully understood.

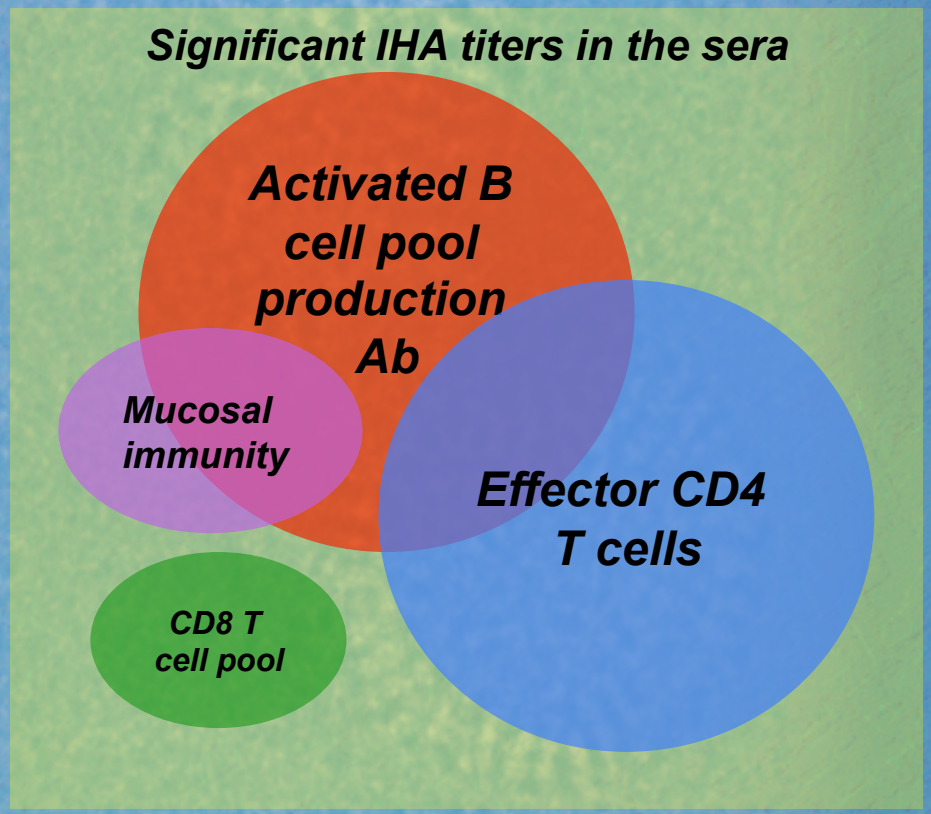
Conclusions. TIV protected against strain-matched infection in children. Seasonal influenza infection appeared to confer cross-protection against pandemic influenza. Whether prior seasonal influenza vaccination affects the risk of infection with the pandemic strain requires additional study.

(See the editorial commentary by Glezen, on pages 1380–1382.)

Hypothesis on multiple vaccination and influenza cell expansion



Influenza VACCINATION/INFECTION



- ✓ **Define the combination of parameters for protection in regard to age and immunological experience status**
- ✓ **Define long term memory responses one year after vaccination with an adjuvanted vaccine**
- ✓ **Comparison to influenza infection ?**
  → memo-FLU-ARDS

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