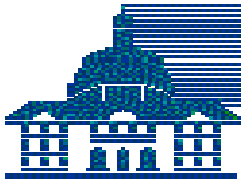
Inserm

Institut national
de la santé et de la recherche médicale



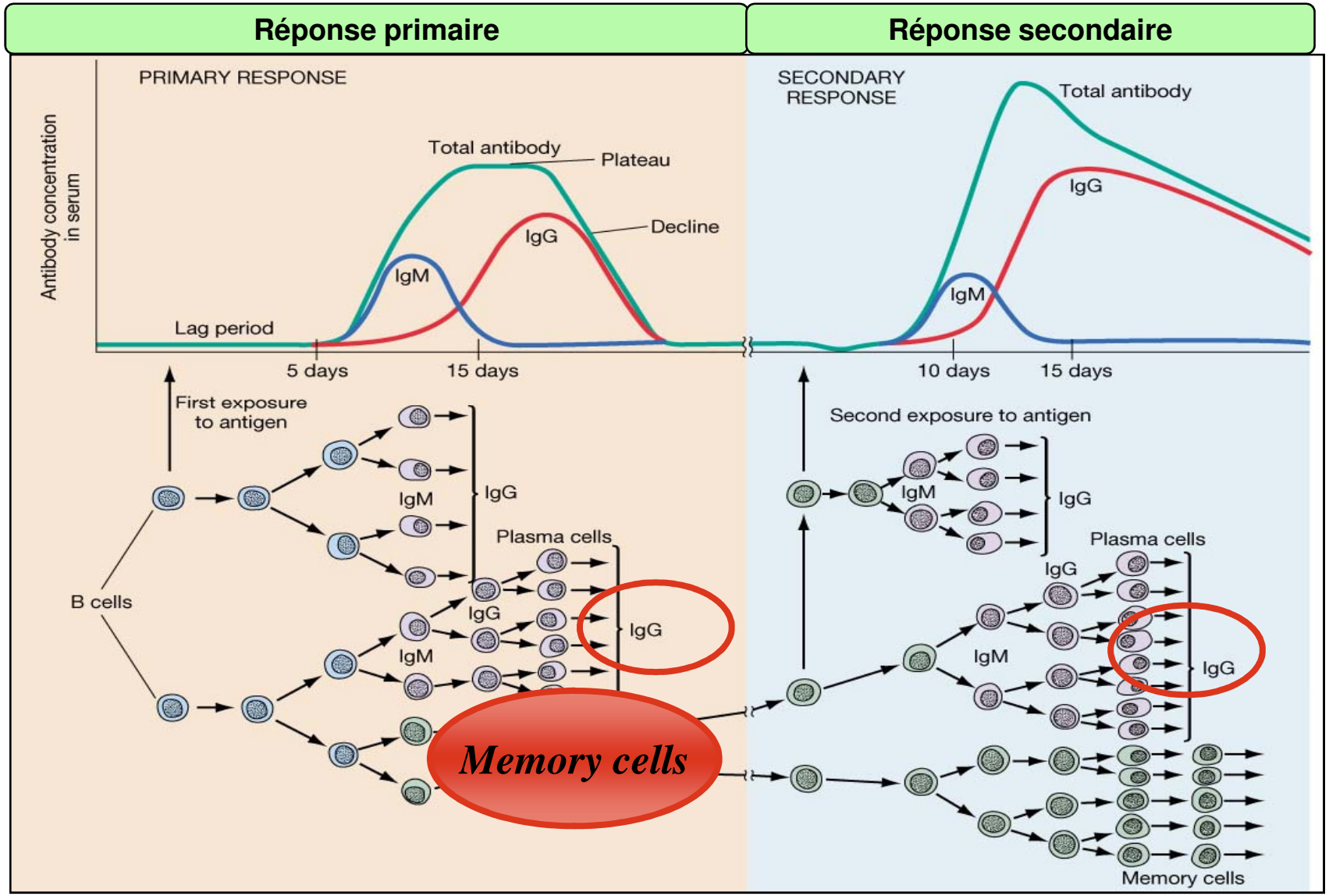
UPMC
PARIS UNIVERSITAS

Mémoire Immunitaire

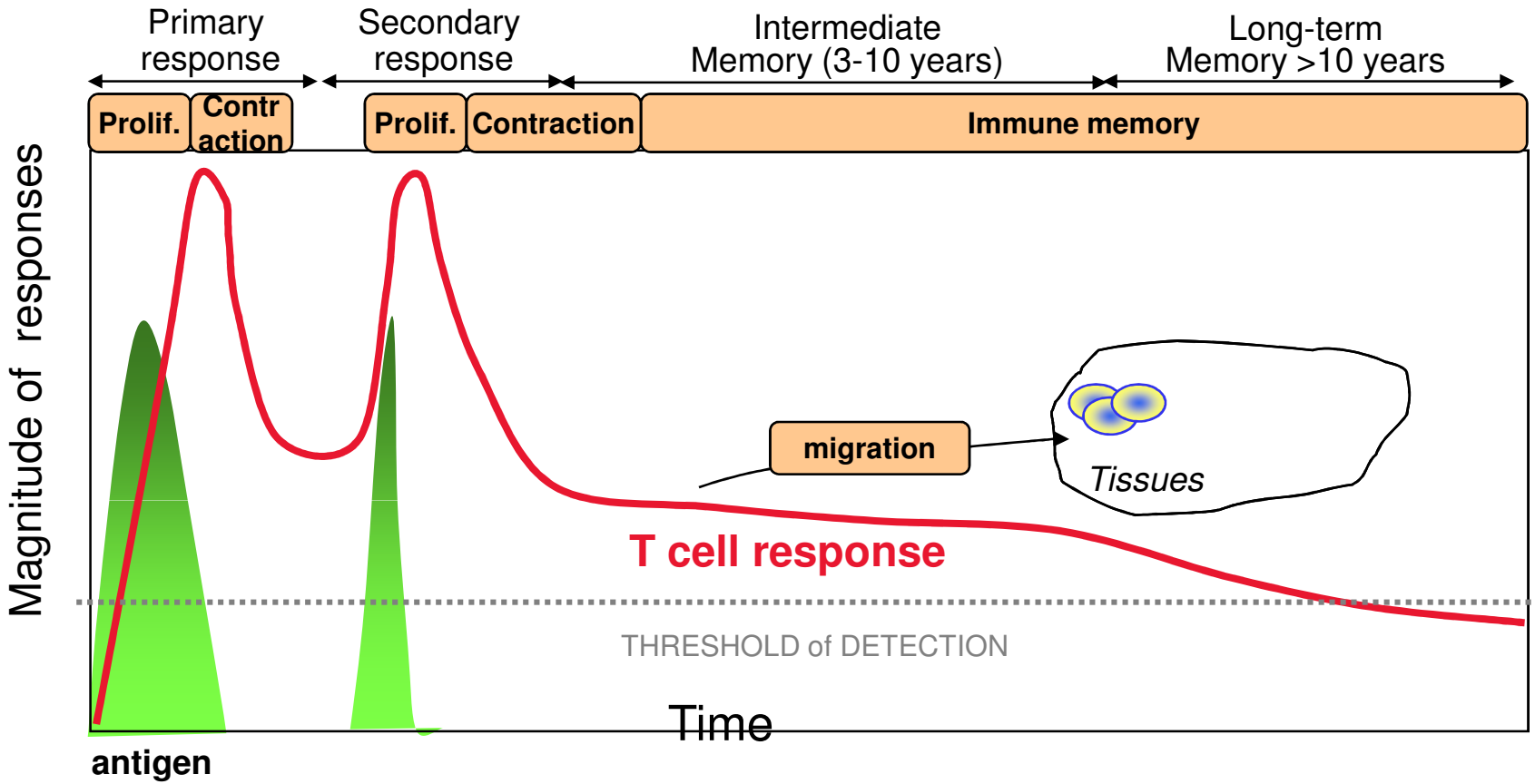
Behazine Combadière,
Directeur de recherche
INSERM

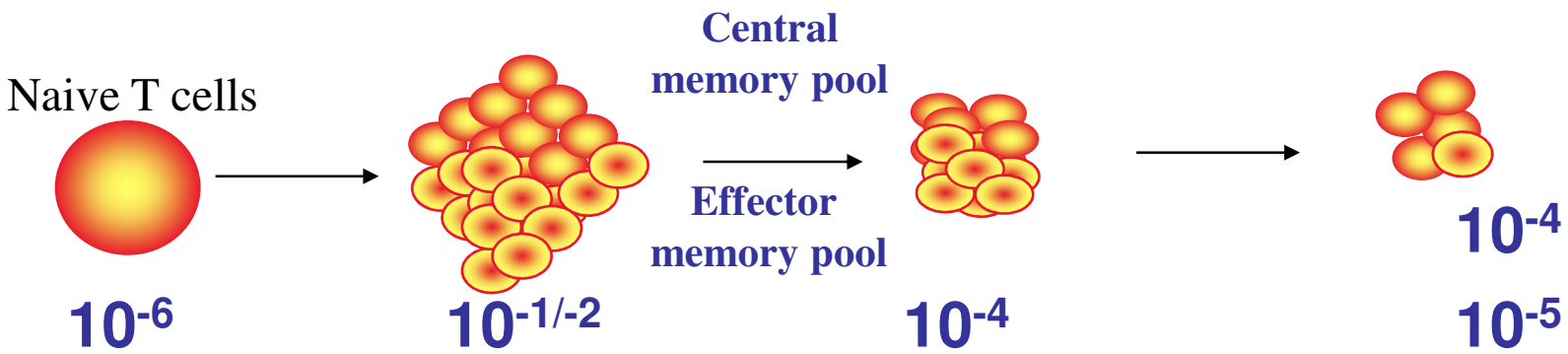
GEIG, Nice 27 novembre 2009

Bcell immune responses and Memory

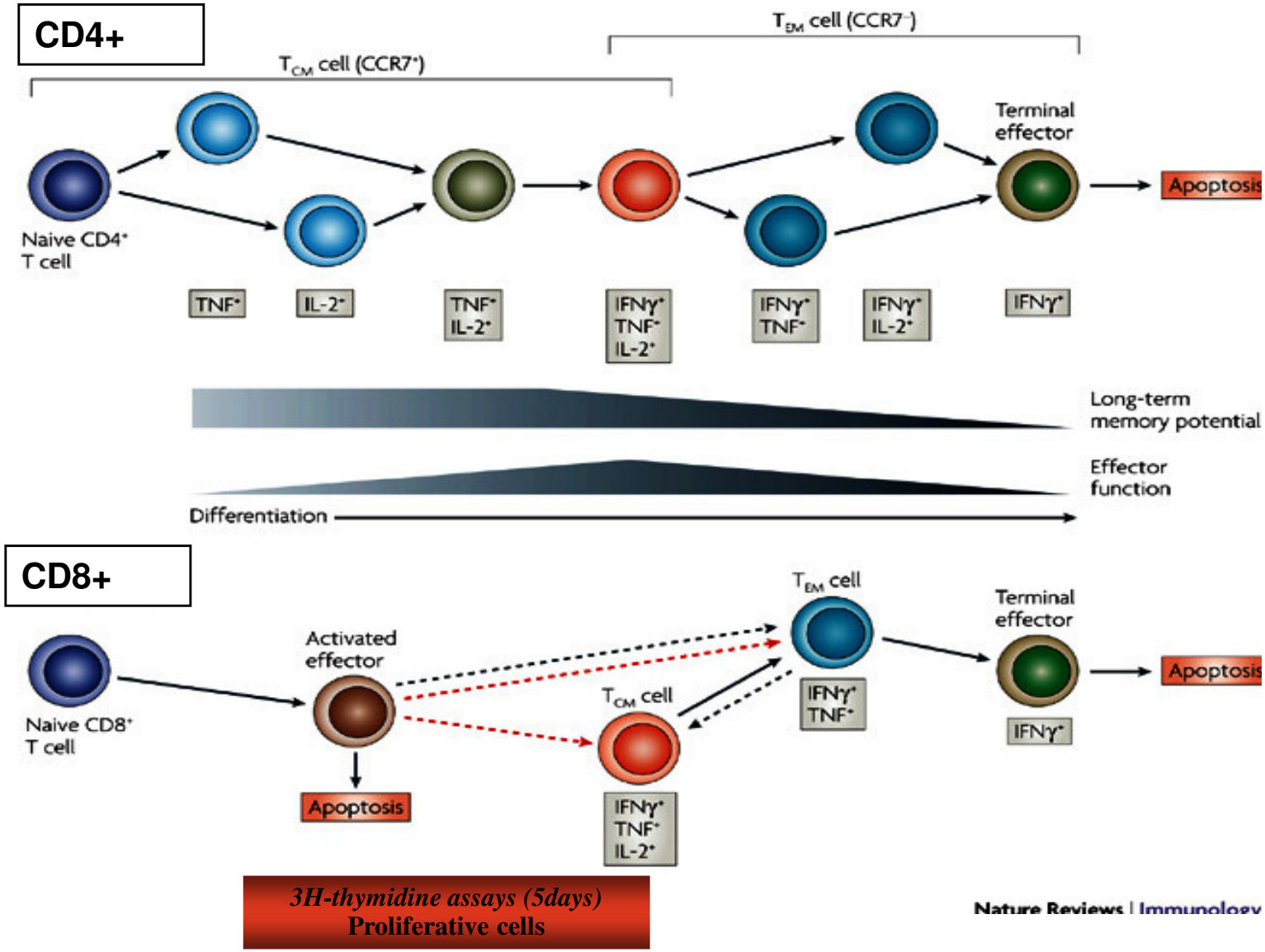


T cell immune responses and Memory





Differentiation of effector /memory T lymphocytes

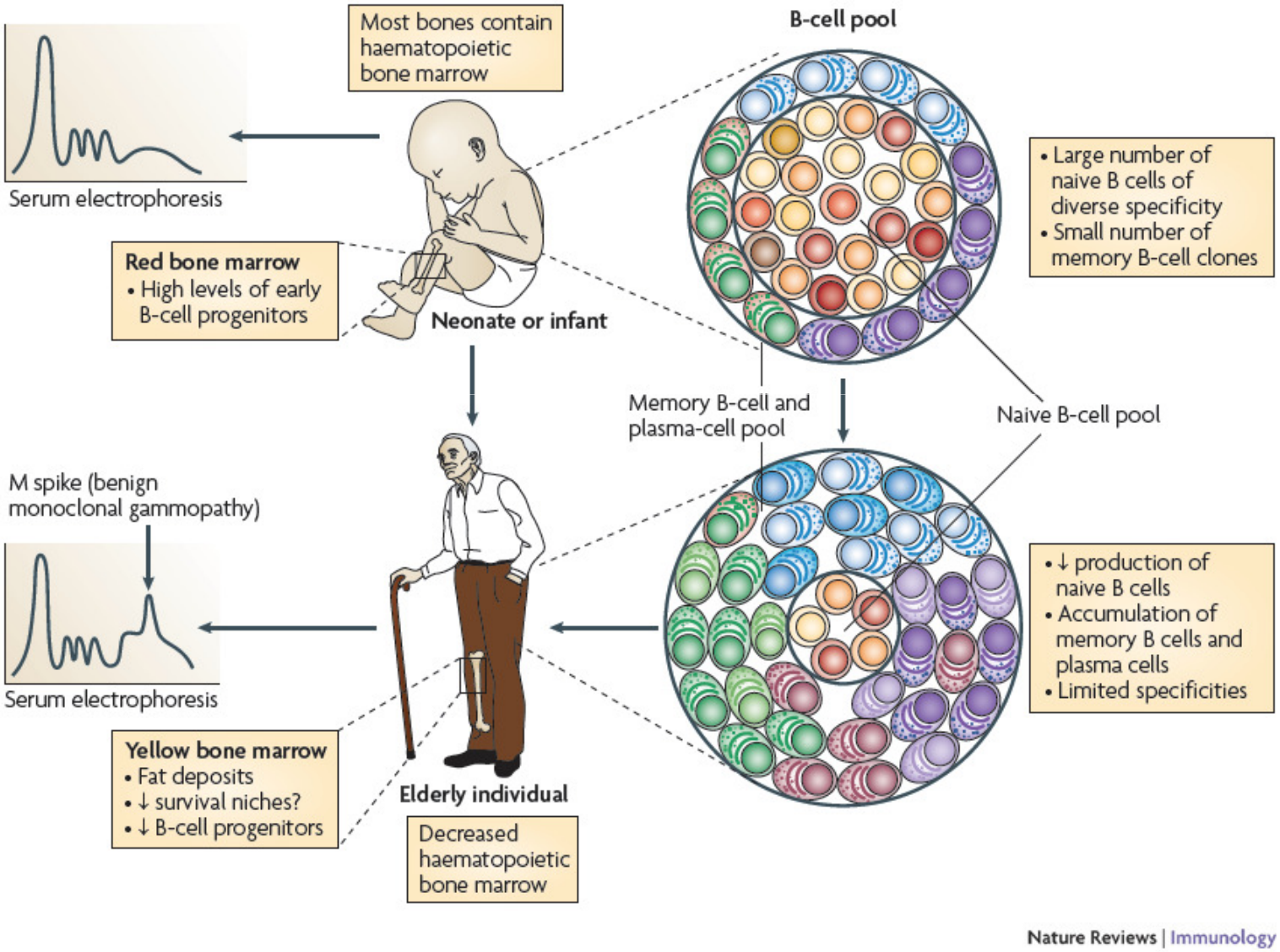


3H-thymidine assays (5days)
Proliferative cells

Elispot / intracellular cytokine staining (ICS) (few hours)
Cytokine-producing cells

Cellules B chez l'enfant et les personnes âgées:

Limites de la réponse immunitaire



Persistance de la réponse mémoire après vaccination?

- ***Virus vivants atténués (persistance de l'antigène)***

  Rougeole, Varicelle

  Les anticorps peuvent persistent jusqu'à 10 ans après la vaccination

- ***Virus vivant (en absence d'antigène circulant)***

  Virus de la vaccine

  Les anticorps peuvent persistent jusqu'à 30 ans après la vaccination mais diminution des taux d'anticorps neutralisants

  Persistance des cellules T mémoires

- ***non-replicatif (persistance faible de l'antigène et faible circulation)***

  tetanos, diphtherie

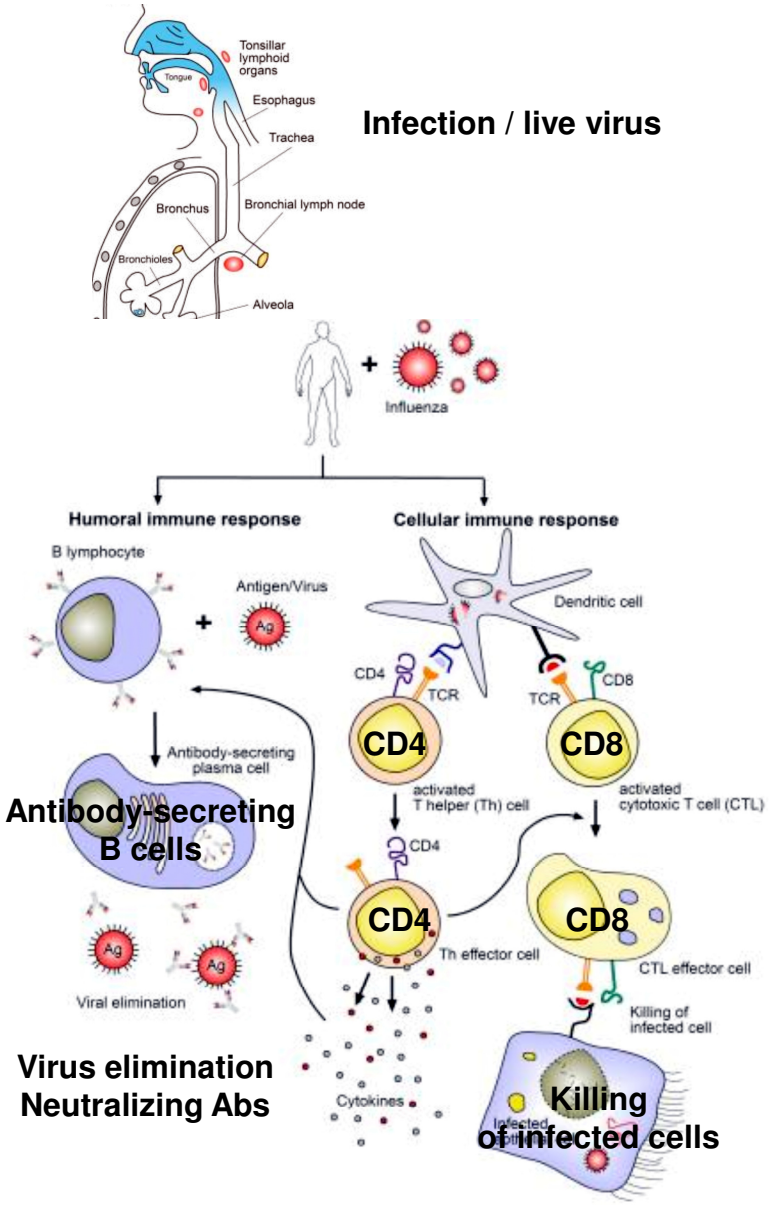
  Les anticorps diminuent après 10 ans

- ***Protéines influenza virales (persistance faible de l'antigène et forte circulation de variants)***

  Persistance des Ac neutralizant spécifique de la souche vaccinale (15-50%)

  Persistance des cellules T spécifques des protéines conservées

influenza virus : Infection



Réponses immunitaires contre le virus d'influenza

✓ **Anticorps neutralisants contre HA et N**

IgG : Protection contre l'infection

IgA

- mutation de HA et Neuraminidase : absence de reconnaissance des Ac (mémoire)

✓ **Immunité cellulaire**

Cellules CD8

Rôle important des cellules CD8 cytotoxique dans l'élimination du virus

Réduction notable de la charge virale

Taux élevés de cellules CD8 : contrôle de l'infection en absence de d'AcN

Limiter l'infection et diminuer sa gravité

Protection chez les personnes âgées

Réactivité croisée des lymphocytes CD8 contre les variants viraux

Reconnaissance des protéines conservées

Cellules CD4

Switch isotypique

Help aux cellules CD8, production de cytokines anti-virales

Réactivité croisée des cellules CD4 --> réactivité croisée des anticorps

Persistance des cellule mémoires à long terme

Influenza vaccines

2009-2010 Northern Hemisphere-Recommended
A/Brisbane/10/2007 (H3N2)-like virus
A/Brisbane/59/2007 (H1N1)-like virus
B/Brisbane/60/2008-like virus
Alternates
A/South Dakota/6/2007 (H1N1)
A/Uruguay/716/2007 (H3N2)
B/Brisbane/33/2008

2008-2009 Northern Hemisphere-Recommended
A/Brisbane/59/2007 (H1N1)-like virus
A/Brisbane/10/2007 (H3N2)-like virus
B/Florida/4/2006-like virus

2007-2008 Northern Hemisphere-Recommended
A/Solomon Islands/3/2006 (H1N1)-like virus
A/Wisconsin/67/2005 (H3N2)-like virus
B/Malaysia/2506/2004-like virus
Alternates
A/Wisconsin/67/2005 (H3N2)
A/Hiroshima/52/2005 (H3N2)

2006-2007 Northern Hemisphere-Recommended
A/New Caledonia/20/1999 (H1N1)-like virus
A/Wisconsin/67/2005 (H3N2)-like virus
B/Malaysia/2506/2004-like virus
Alternates
A/Wisconsin/67/2005 (H3N2)
A/Hiroshima/52/2005 (H3N2)
B/Malaysia/2506/2004
B/Ohio/1/2005

2005-2006 Northern Hemisphere-Recommended
A/New Caledonia/20/1999 (H1N1)-like virus
A/California/7/2004 (H3N2)-like virus
B/Shanghai/361/2002-like virus
Alternates
A/New York/55/2004 (H3N2)
B/Shanghai/361/2002
B/Jiangsu/10/03
B/Jilin/20/2003

2004-2005 Northern Hemisphere-Recommended
A/New Caledonia/20/1999 (H1N1)-like virus
A/Fujian/411/2002 (H3N2)-like virus
B/Shanghai/361/2002-like virus
Alternates
A/Wyoming/03/2003 (H3N2)
A/Kumamoto/102/2002 (H3N2)
B/Jilin/20/2003

2003-2004 Northern Hemisphere-Recommended
A/New Caledonia/20/1999 (H1N1)-like virus
A/Moscow/10/99 (H3N2)-like virus
B/Hong Kong/330/2001-like virus
Alternates
A/Panama/2007/1999 (H3N2)
B/Shandong/7/97
B/Hong Kong/330/2001
B/Hong Kong/1434/2002

2002-2003 Northern Hemisphere-Recommended
A/New Caledonia/20/1999 (H1N1)-like virus
A/Moscow/10/99 (H3N2)-like virus
B/Hong Kong/330/2001-like virus
Alternates
A/Panama/2007/1999 (H3N2)

2001-2002 Northern Hemisphere-Recommended
A/New Caledonia/20/1999 (H1N1)-like virus
A/Moscow/10/99 (H3N2)-like virus
B/Sichuan/379/99-like virus
Alternates
A/Panama/2007/1999 (H3N2)
B/Victoria/504/2000
B/Johannesburg/5/99

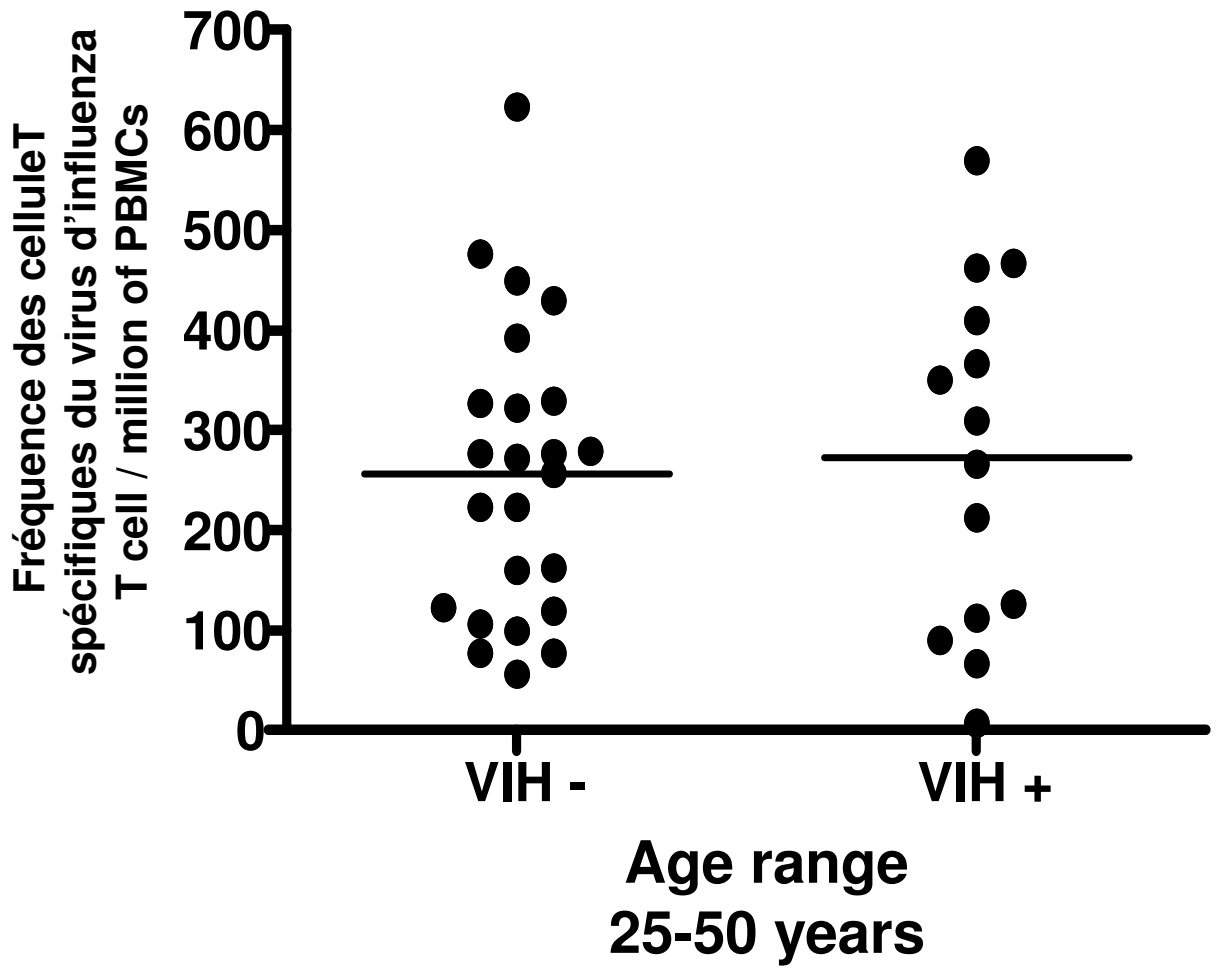
2000-2001 Northern Hemisphere-Recommended
A/New Caledonia/20/1999 (H1N1)-like virus
A/Moscow/10/99 (H3N2)-like virus
B/Beijing/184/93-like virus
Alternates
A/Panama/2007/1999 (H3N2)
B/Yamanashi/166/98

1999-2000 Northern Hemisphere-Recommended
A/Sydney/5/97 (H3N2)-like virus
A/Beijing/262/95 (H1N1)-like virus
B/Beijing/184/93-like virus
Alternates
B/Shangdong/7/97
B/Harbin/7/94

1998-1999 Northern Hemisphere-Recommended
A/Beijing/262/95 (H1N1)-like virus
A/Sydney/5/97 (H3N2)-like virus
B/Beijing/184/93-like virus
Alternates
B/Harbin/7/94

(2004-2005 flu vaccine)-specific memory T cell responses

24 sujets sains et 12 sujets HIV inclus dans la cohorte Manon 05 : vaccin 2004-2005
--> analyse des réponses mémoires cellulaires avant vaccination



Combadière et al.

Analysis of cellular immune responses

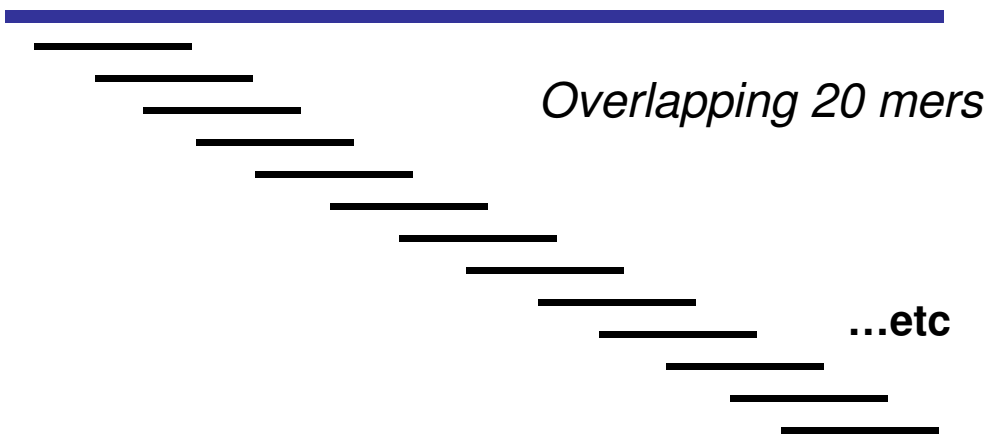
SURFACE PROTEIN : HAEMAGLUTININ

- ***H1*** *(A/NEW CALEDONIA/20/99)*
- ***H3*** *(A/CALIFORNIA/7/2004)*

CORE PROTEIN : NUCLEOPROTEIN

- ***NP*** *(A/NEW CALEDONIA/20/99)*

(≈98% homology between strains)

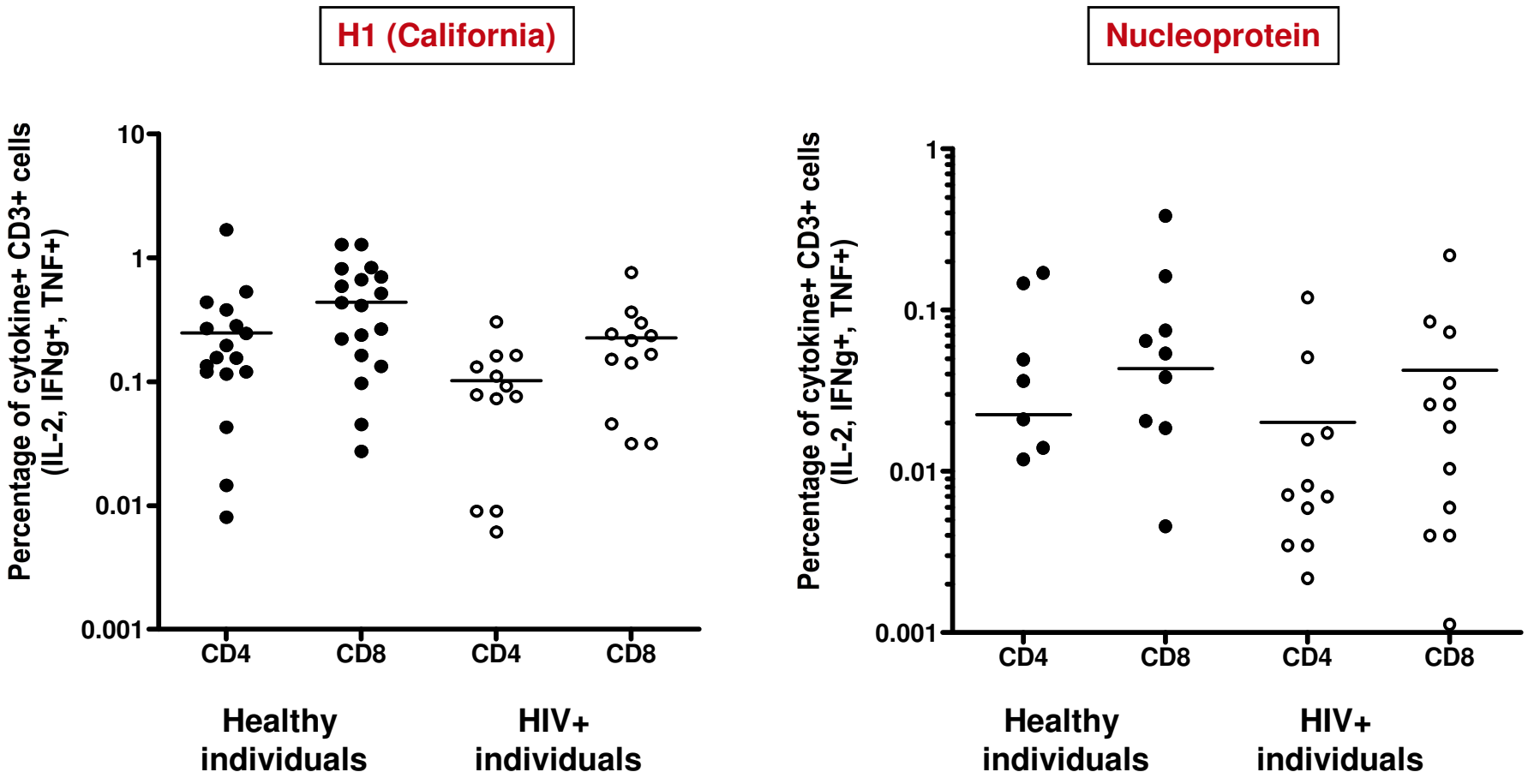


Method:

- *in vitro* stimulation of fresh PBMCs with overlapping 20mers of influenza for 24 hours
- Multiparametric flow cytometric analysis: CD3, CD4, CD8, CD27, CD45RA, CD11a, CCR7, CCR5, IL-2, TNFα, IFNγ··LSRII)

ANALYSIS OF CTYOKINE PRODUCTION BY T CELLS

influenza-specific memory T cell respones



T cell epitopes of Influenza proteins

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

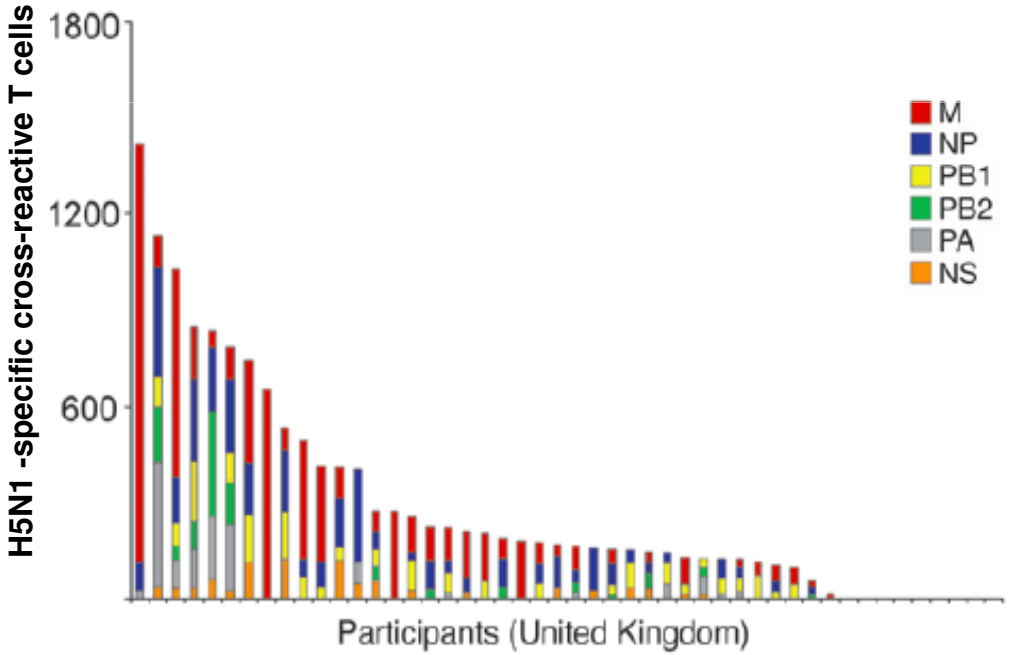
The total number of epitopes in the H1N1 seasonal flu strains from 1988–2008 (Total) as well as the number of epitopes conserved in swine-origin H1N1 influenza virus (S-OIV) (Cons.) are listed.

 Related Commentary, page 3273

Memory T cells established by seasonal human influenza A infection cross-react with avian influenza A (H5N1) in healthy individuals

Laurel Yong-Hwa Lee,[1] Do Lien Anh Ha,[2] Cameron Simmons,[2] Menno D. de Jong,[2] Nguyen Van Vinh Chau,[2] Reto Schumacher,[1] Yan Chun Peng,[1] Andrew J. McMichael,[1] Jeremy J. Farrar,[2] Geoffrey L. Smith,[3] Alain R.M. Townsend,[4] Brigitte A. Askonas,[1] Sarah Rowland-Jones,[1] and Tao Dong[1]

[1]MRC Human Immunology Unit, Weatherall Institute of Molecular Medicine, University of Oxford, John Radcliffe Hospital, Oxford, United Kingdom. [2]Oxford University Clinical Research Unit, Hospital for Tropical Diseases, Ho Chi Minh City, Viet Nam. [3]Department of Virology, Faculty of Medicine, Imperial College London, London, United Kingdom. [4]Molecular Immunology Group, Weatherall Institute of Molecular Medicine, University of Oxford, John Radcliffe Hospital, Oxford, United Kingdom.



Humoral vs Cellular cross-reactive immune responses

Informatic tools for prediction of T cell epitopes of influenza strains. De Groot et al. Vaccine 2009

- *Heterogeneity of Hemaglutinin and Neuraminidase = lack of antibody responses*

1. Amino-acid identity ≈ 75% homology --> lack of cross-reactive antibodies
2. Variation in H1 sequence during epidemics can account in some cases for conventional vaccine failure

- *Conserved T cell epitopes for CD4 and CD8 responses based on prediction of epitopes using bio-informatic tools*

1. Good cross-reactivity of CD4+ (MHC-Class II restriced) helper cells

2. Fair to good cross-reactivity of CD8+ (MHC-Class I restricted) cytotoxic cells
3. Less cross-reactivity against neuraminidase

Cellular cross-reactive immune responses

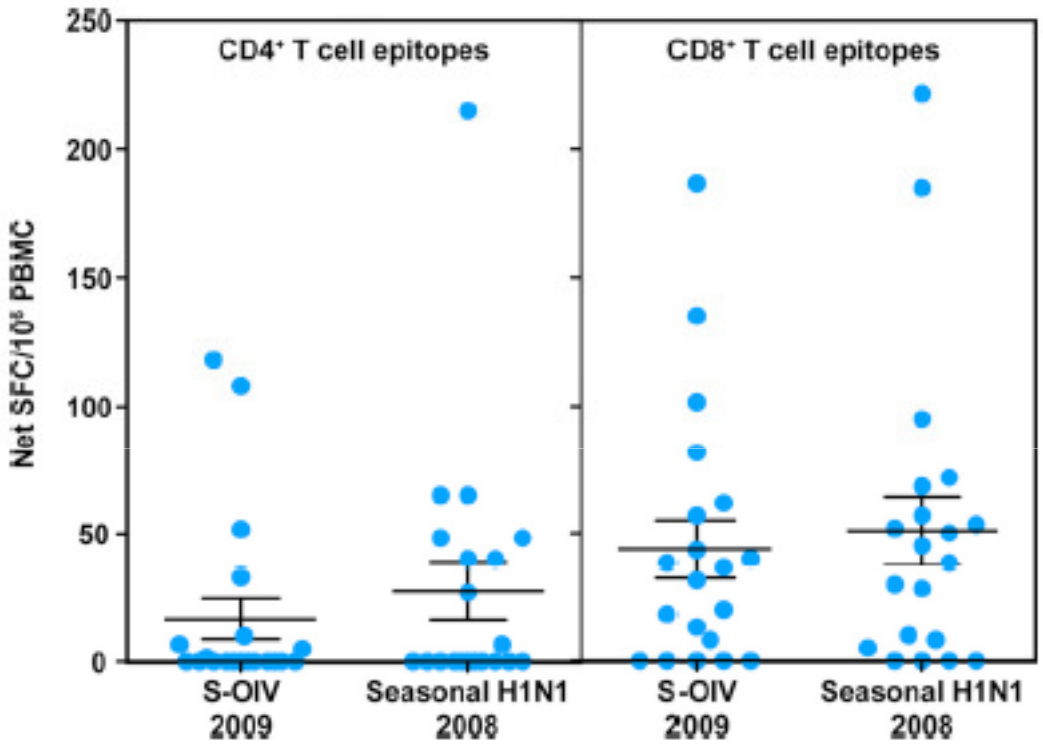
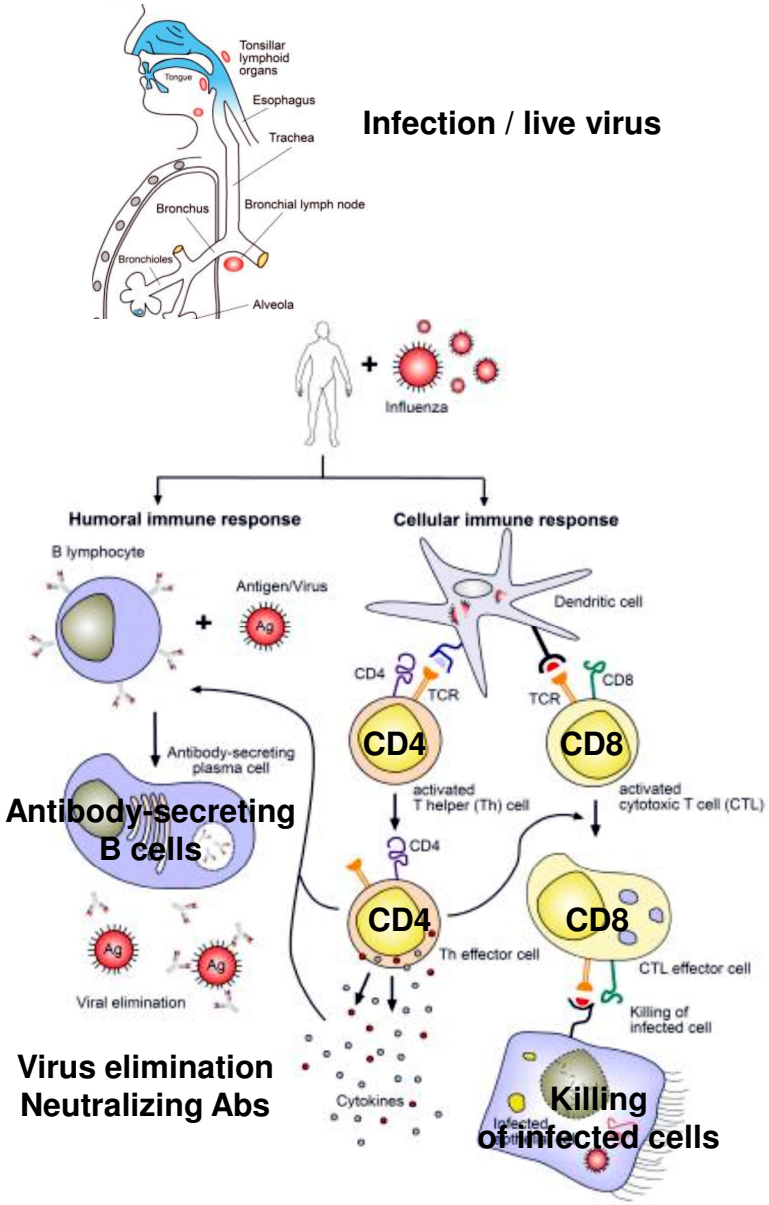
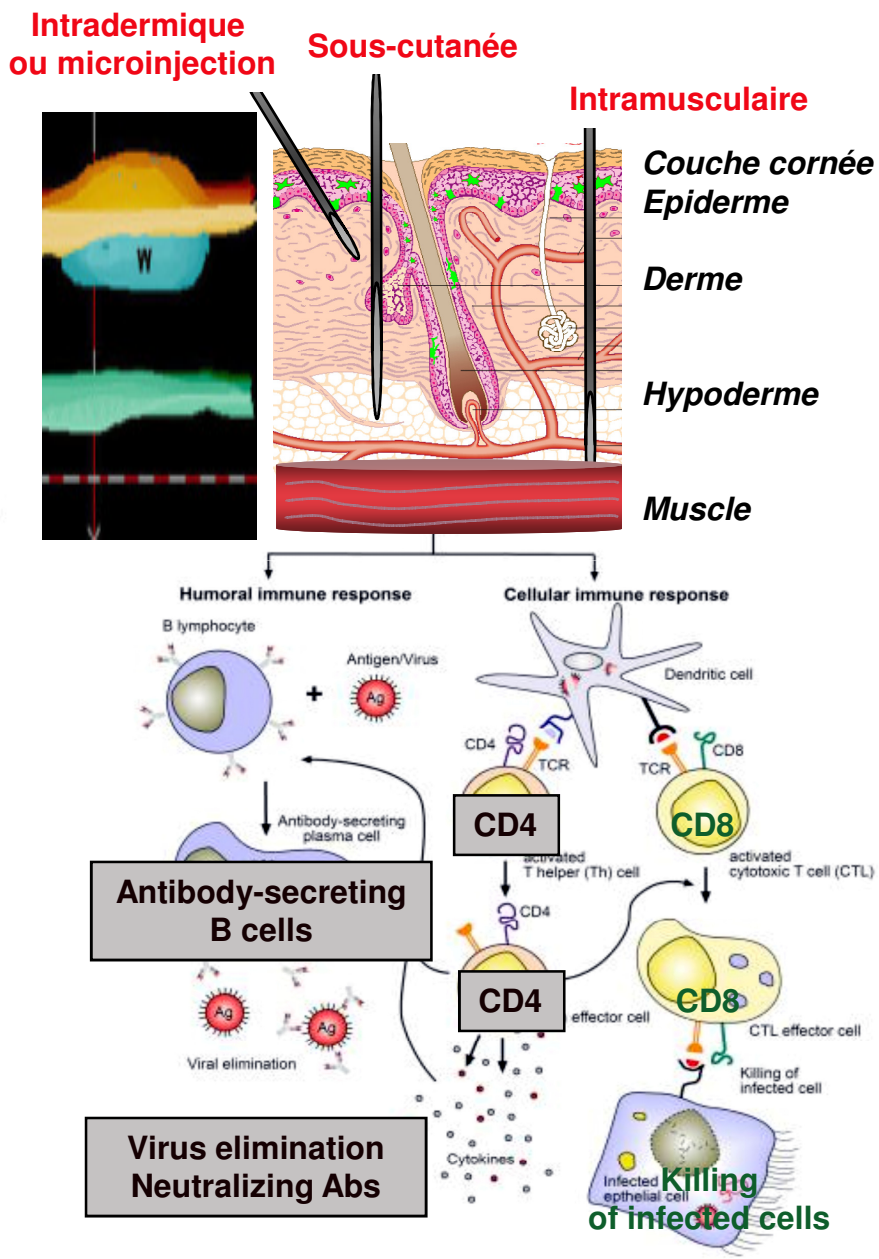


Fig. 4. Comparable pre-existing CD4+ and CD8+ T-cell immunity to S-OIV and 2008 seasonal influenza. PBMC from normal individuals ($n = 20$) were stimulated with pools of either CD4+ or CD8+ T-cell epitopes that were conserved in S-OIV or seasonal influenza 2008 sequences. Responses were measured through ex vivo IFN-γ ELISPOT assays. Error bars represent SEM.

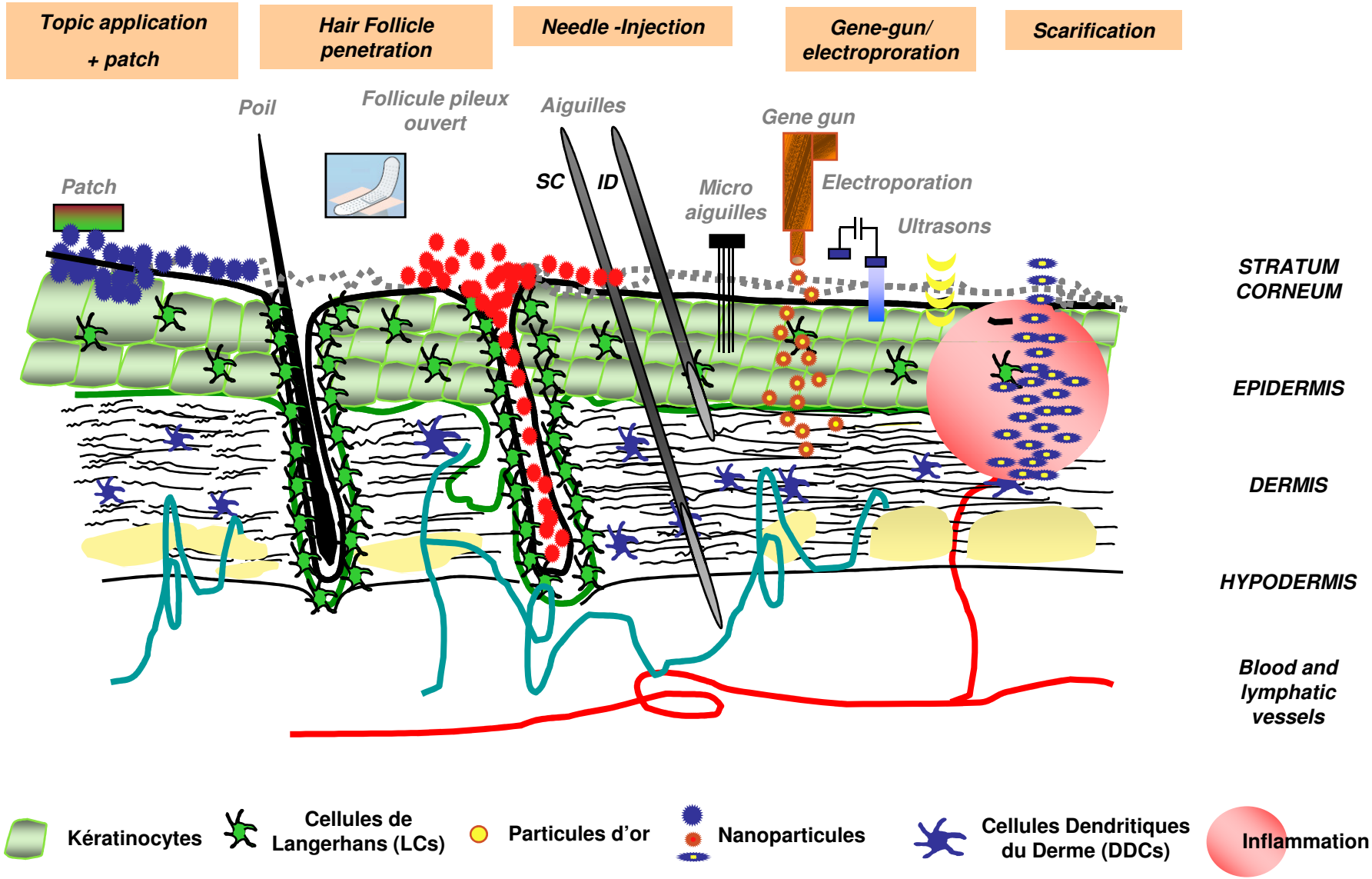
Greenbaum et al. PNAS 2009

influenza virus : *Infection vs vaccination*





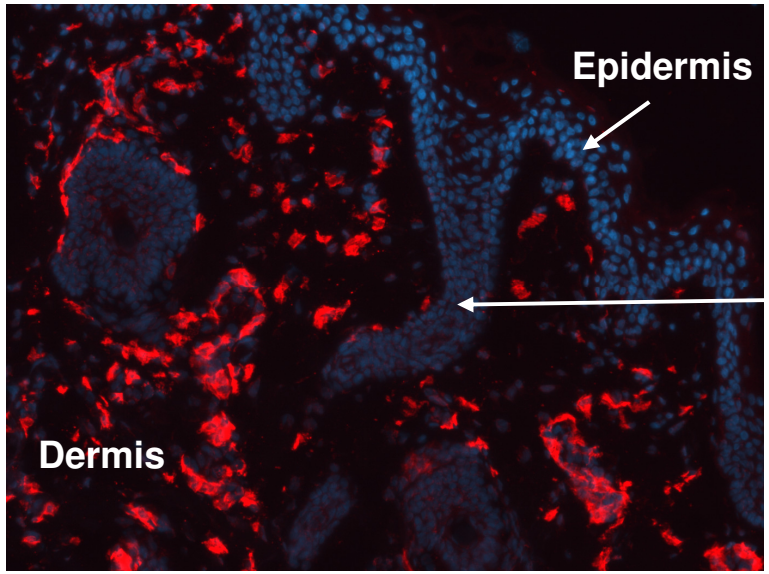
Skin routes of immunization



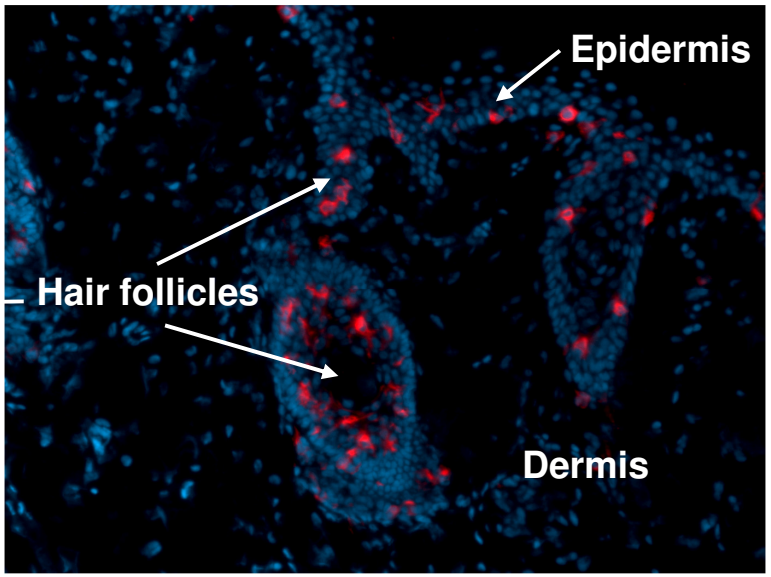
Rational : skin' routes of vaccination

- The high concentration of various dendritic cells in the skin has been prompting numerous studies of epidermal or dermal immunization. Langerhans cells (LCs, CD207+, CD1a+ CD14-) in the epidermis and DCs (CD205, CD14+CD1a- and langerin+/-) in the dermis *(Kupper. Nat Rev Immunol. 2004)*
- In mice models: targeting antigens to LCs is more efficient than DDCs at priming and cross-priming highly cytotoxic CD8 Tcells *(Valladeau, 2005, Klechevsky, 2008)*

Human skin explants



CD209 (Dermal Dendritic cells)
DAPI (cell nucleus)



CD1a (Langerhans cells)
DAPI (cell nucleus)

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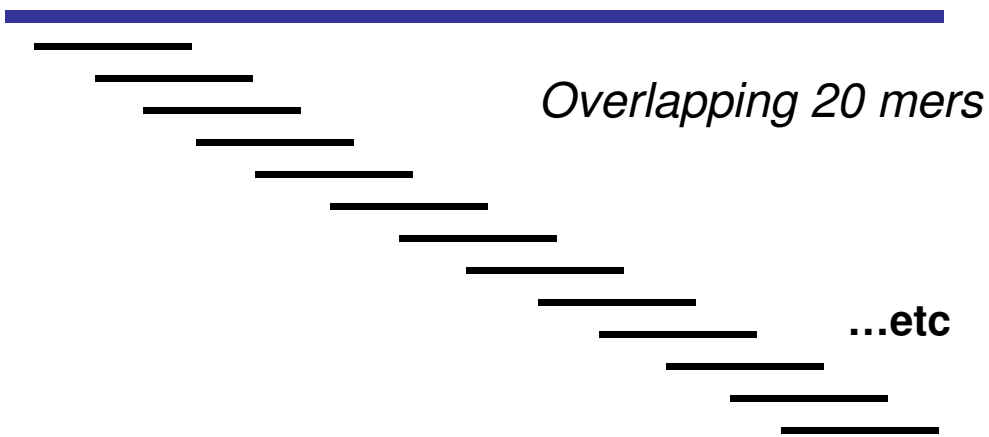

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ANALYSIS OF CTYOKINE PRODUCTION BY T CELLS

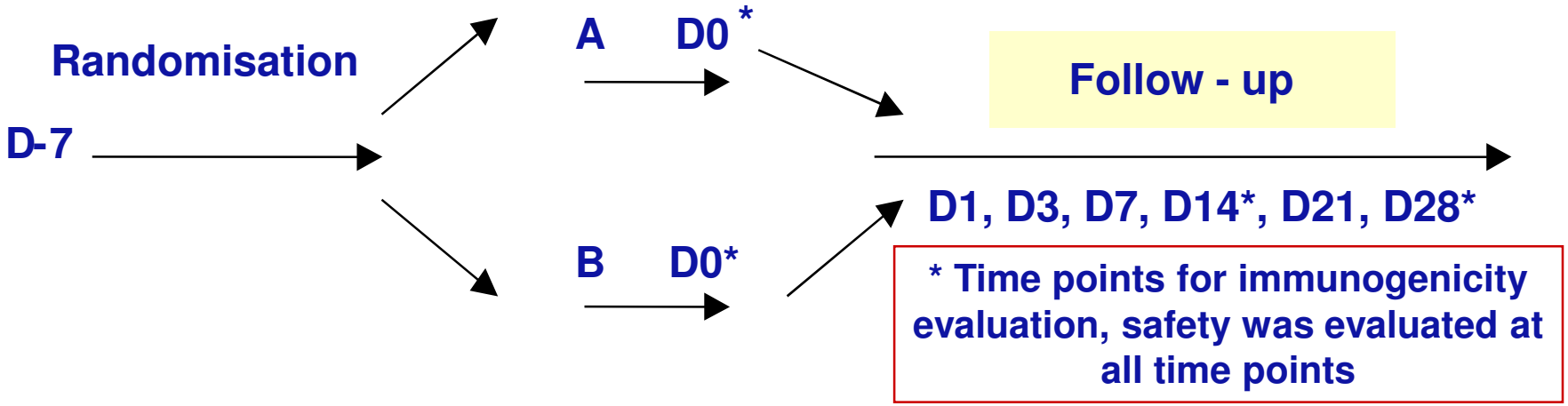
Study design : MANON 05

TETAGRIP®
SANOFI PASTEUR MSD

2005/2006

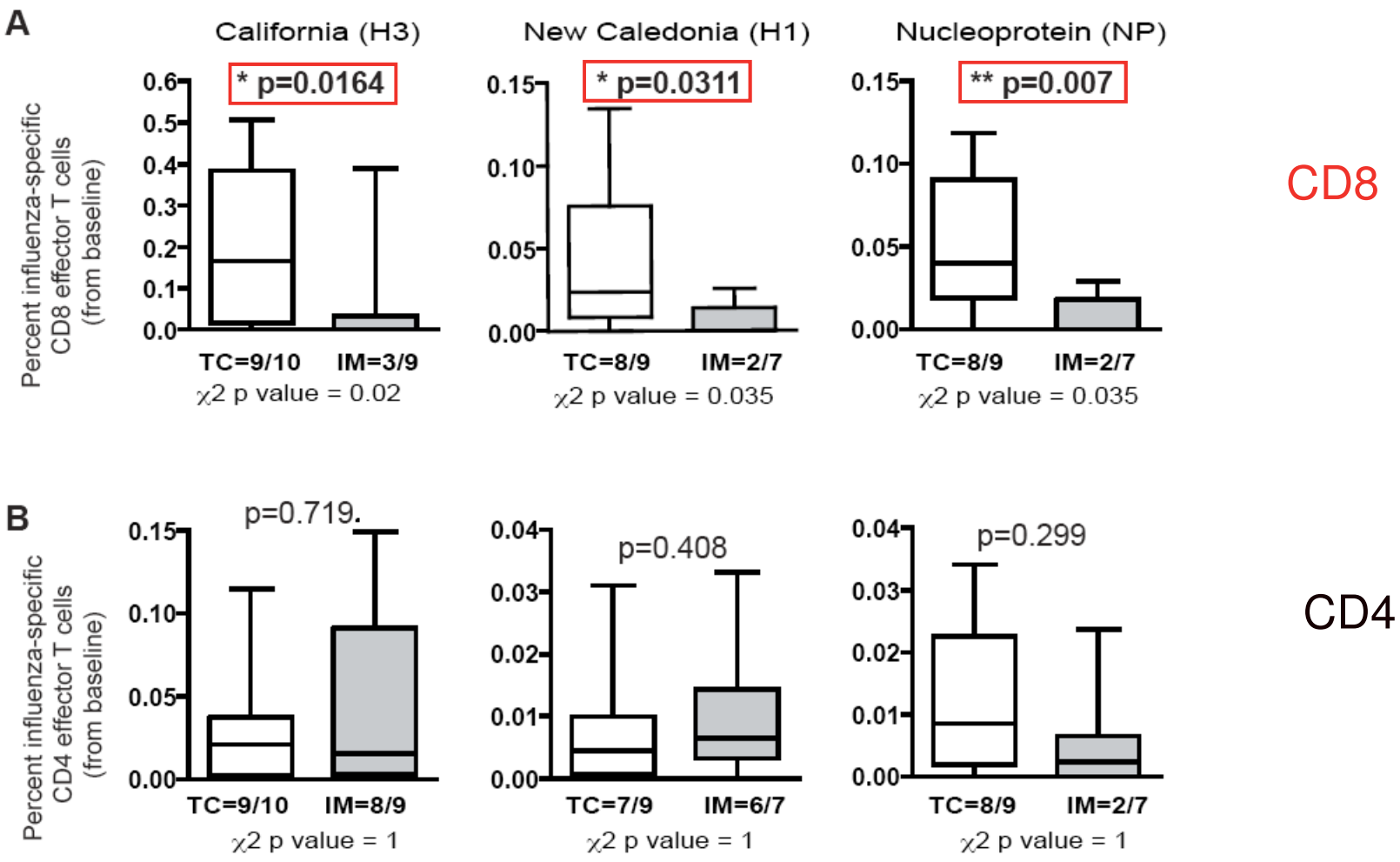
A/CALIFORNIA/7/2004 (**H3**N2)	*NYMC X-157*
	A/NEW YORK/55/2004
A/NEW CALEDONIA/20/99 (**H1**N1)	*IVR-116*
B/SHANGAI/361/2002	*B/JIANGSU/10/2003*

Cohort I:	24 Healthy Volunteers (Berlin)
	Two arms (12 TC + 12 IM)
Cohort II:	13 HIV+ patients (Frankfurt)
	Two arms (6 TC + 7 IM)



ORVACS

Preferential induction of CD8 cellular responses by TC route



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Combadière et al PLOSONE

Conclusion

La mémoire immunitaire a besoin d'être stimulée chez l'adulte et chez les personnes âgées

- Rappel en fonction des tranches d'âge

- Développer les qualités intrinsèques du vaccin : composition, choix d'antigène (variabilité, protéines conservées) afin d'induire toutes les armes de l'immunité pour une meilleure protection

- Evaluer l'efficacité des réponses immunitaires après vaccination : les réponses anticorps ne sont pas les seuls composants de la réponse immunitaire

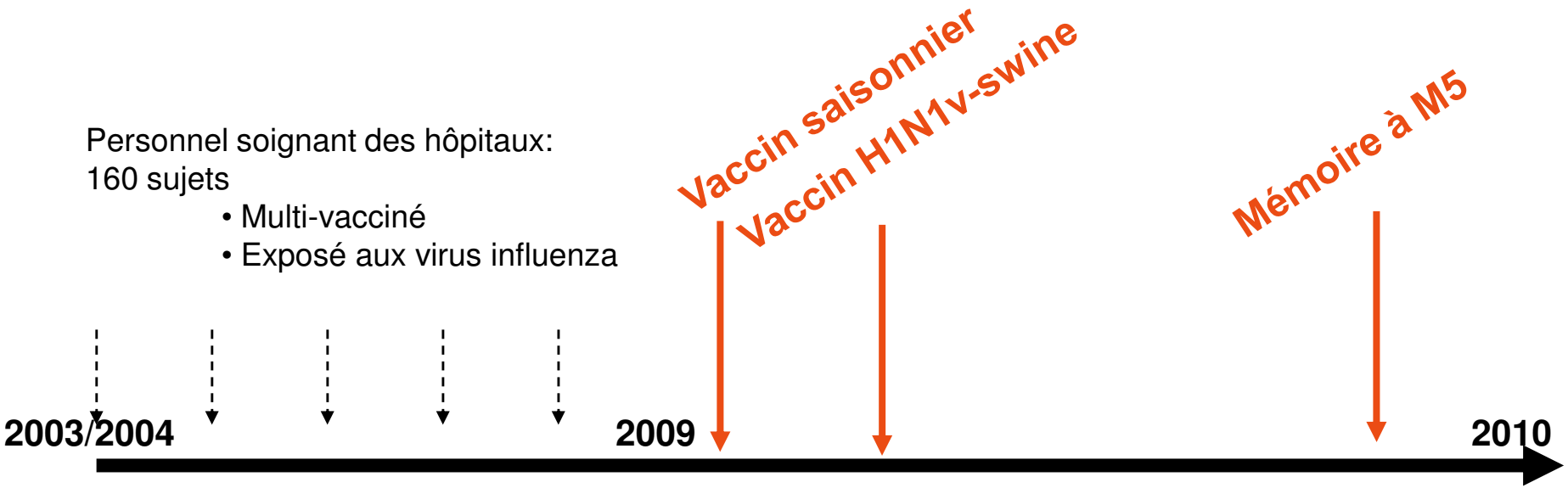
- Evaluer et définir la réactivité vis-à-vis des variants antigéniques

Questions :

- adutles : mieux vaccinés (rappel) --> personnes âgées mieux immunisées ?

- bénéfice/risque immununologique des re-vaccinations multiples ?

Influenza vaccination personnel hospitalier : FLU-HOP



--> Historique (médecine du travail)

- **Des vaccinations antérieures depuis 5 ans**
- **Arrêt de travail : grippe confirmée**
- **Contact avec les malades (personnel des urgences)**

Représentation des différentes tranches d'âge adulte 20 à 65 ans

Réponse mémoire immunitaire humorale et cellulaire?

Réactivité croisée Ac et cellules T

Mémoire vaccinale

Benefice/risque immunologique de la vaccination

Service des maladies infectieuses (Pitié)
Médecine du travail (Pitié)
Centre d'investigation clinique (Cochin)