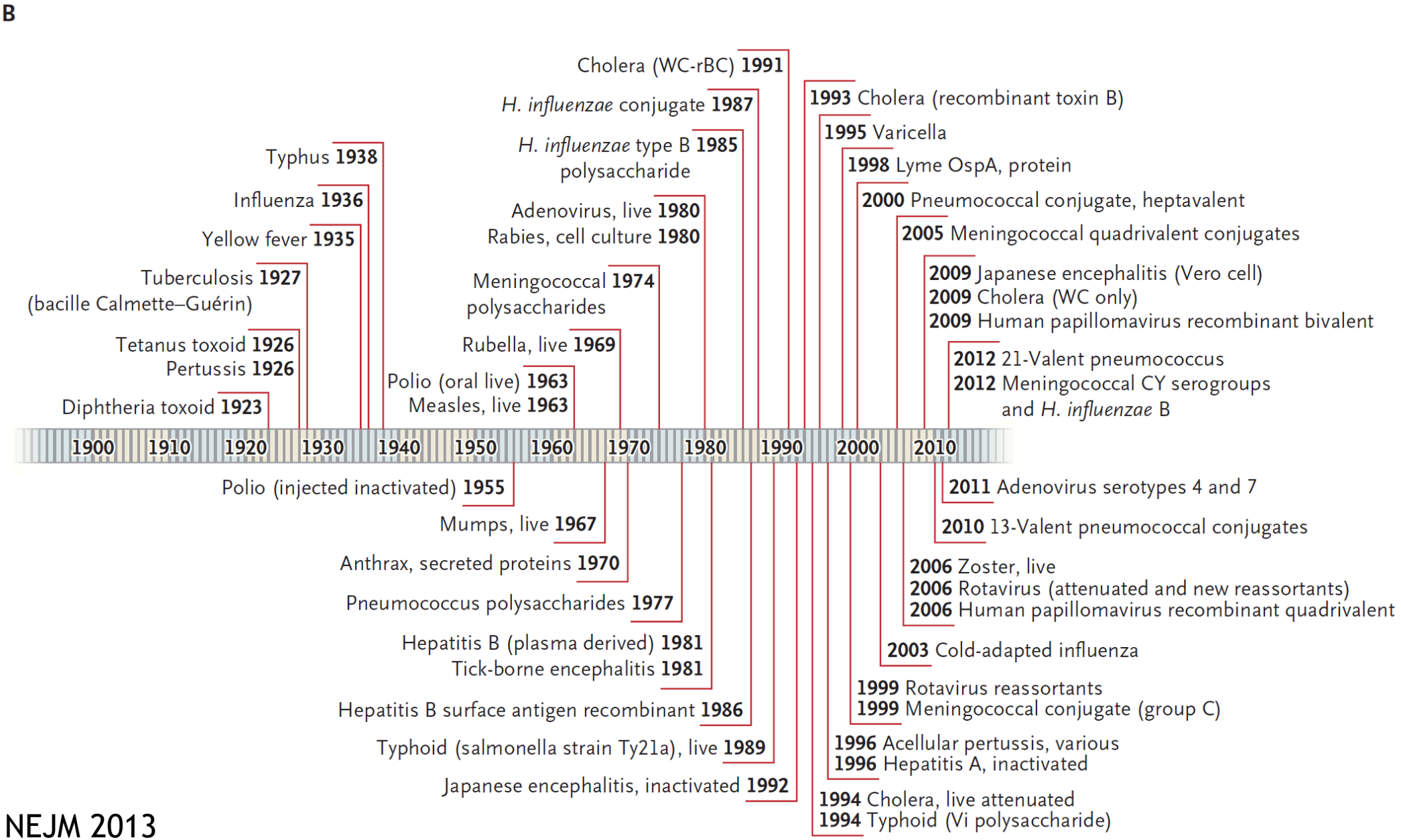
Immunological Challenges in vaccination and vaccine development

Andrew J Pollard FMedSci

What did immunology ever do for vaccines??



Nabel, NEJM 2013

What did we learn about vaccines from immunology?

Correlates for paediatric vaccines

Antibody correlates	Cell mediated correlates (T cell)
Diphtheria, antibody (toxin neutralisation)	
Tetanus, antibody (toxin neutralisation)	
Hib, antibody (ELISA)	
MenC, antibody (serum bactericidal assay)	
Pneumococcus, antibody (ELISA)	
Hepatitis A, antibody (ELISA)	
Hepatitis B, antibody (ELISA)	
Measles, antibody (microneutralisation)	
Rubella, antibody (immunoprecipitation)	
Varicella, antibody (serum neutralisation or gp ELISA)	
Influenza, antibody (HAI)	
Polio antibody (serum neutralisation)	
Rabies, antibody (serum neutralisation)	

Antibody is used to derive quantitative correlates

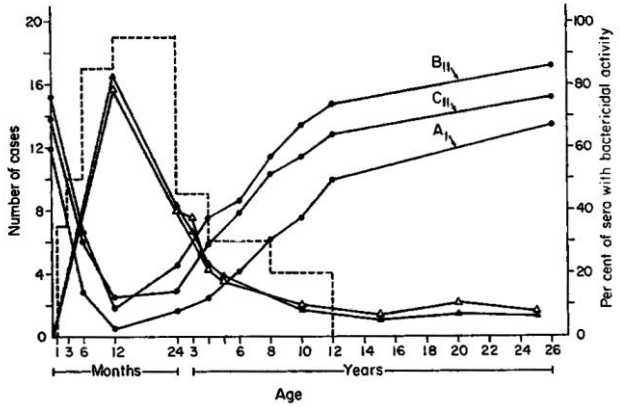
Table 4. Some quantitative correlates of protection after vaccination.

Vaccine	Test	Correlate of protection	Reference(s)
Diphtheria	Toxin neutralization	0.01–0.1 IU/mL	[14]
Hepatitis A	ELISA	10 mIU/mL	[15]
Hepatitis B	ELISA	10 mIU/mL	[16]
Hib polysaccharides	ELISA	1 mcg/mL	[17]
Hib conjugate	ELISA	0.15 mcg/mL	[18]
Influenza	HAI	1/40 dilution	[19]
Lyme	ELISA	1100 EIA U/mL	[20]
Measles	Microneutralization	120 mIU/mL	[7]
Pneumococcus	ELISA; opsonophagocytosis	0.20–0.35 mcg/mL (for children); 1/8 dilution	[21, 22]
Polio	SN	1/4–1/8 dilution	[23]
Rabies	SN	0.5 IU/mL	[24]
Rubella	Immunoprecipitation	10–15 mIU/mL	[25, 26]
Tetanus	Toxin neutralization	0.1 IU/mL	[27]
Varicella	SN; gpELISA	≥1/64 dilution; ≥5 IU/mL	[28, 29]

NOTE. gp, glycoprotein; HAI, hemagglutination inhibition; Hib, *Haemophilus influenzae* type b; SN, serum neutralization.

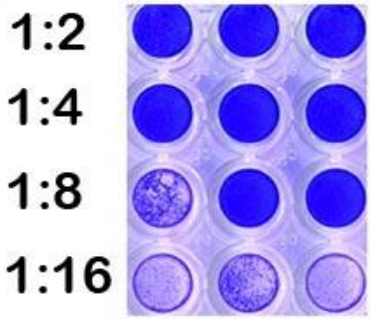
Plotkin CID 2008

Understanding antibody function mechanisms helps identify potential candidates

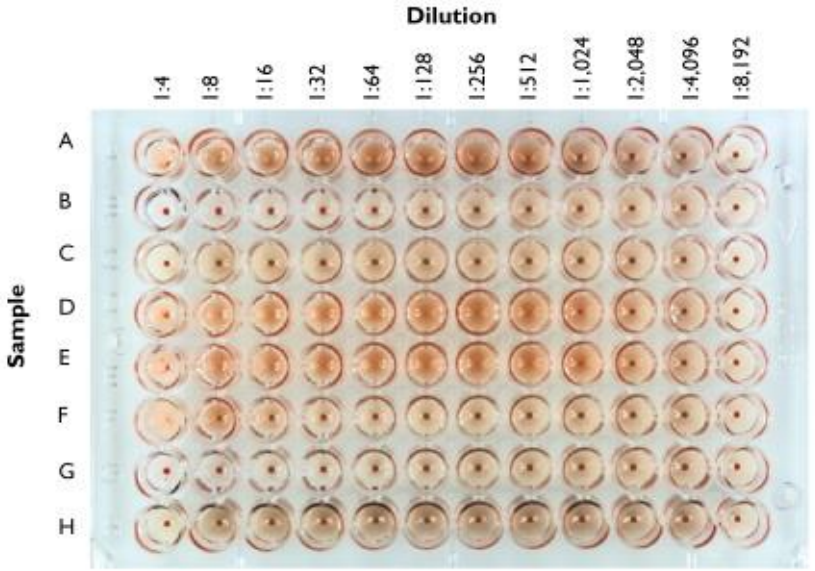


Goldschneider 1969

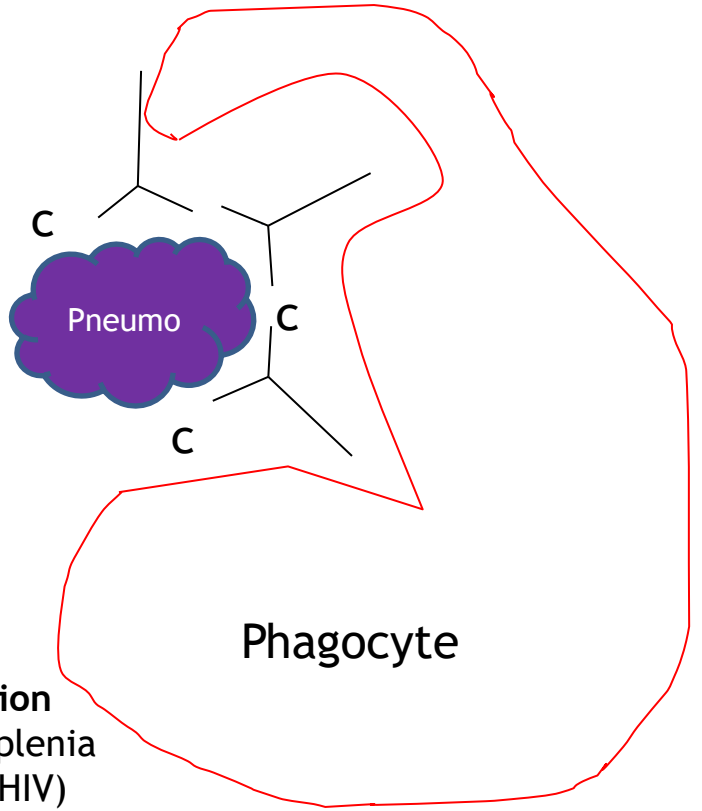
Meningococcal infection
Complement deficiency



microneutralisation

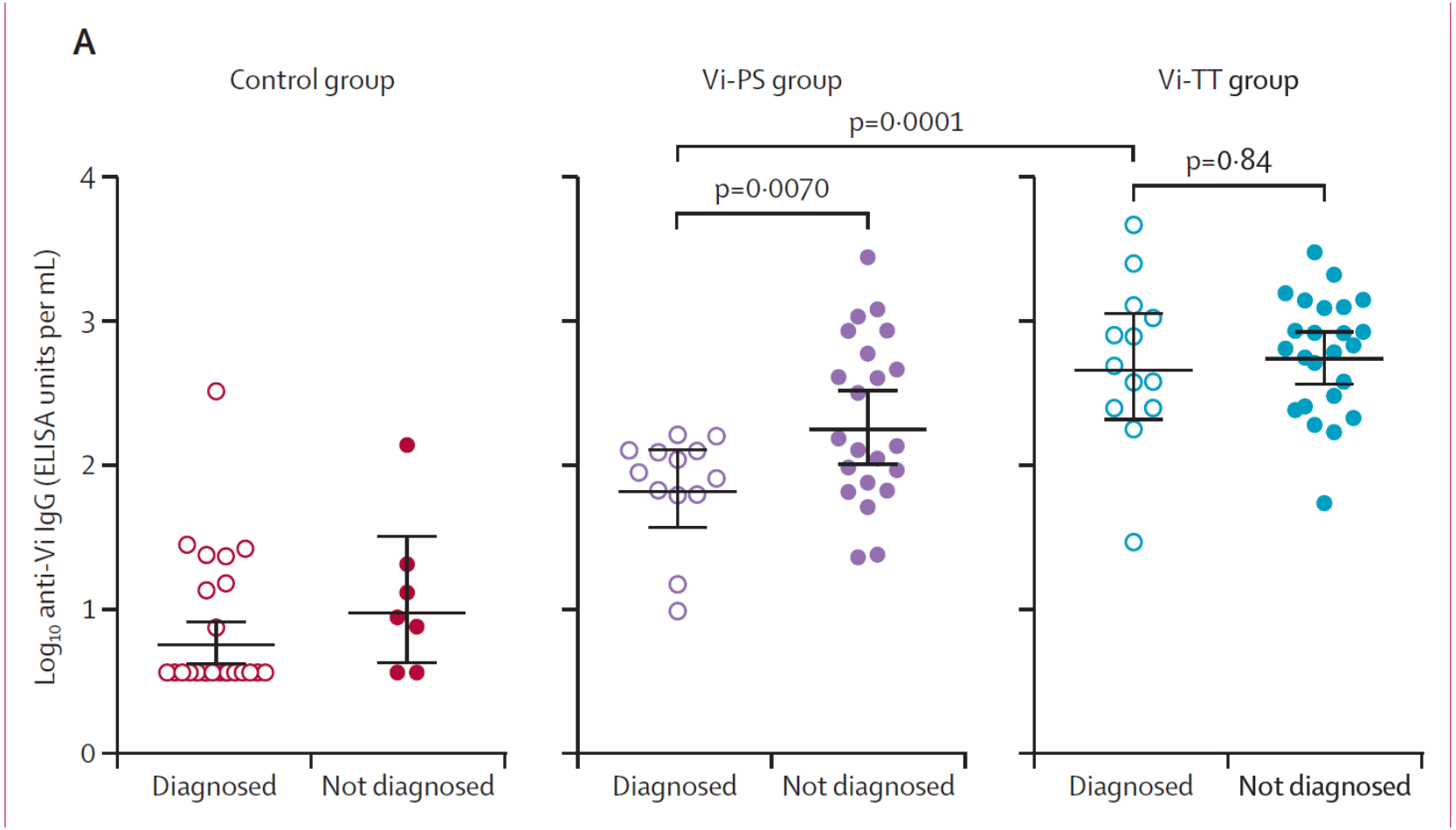


Influenza
HAI (haemaglutination inhibition assay)

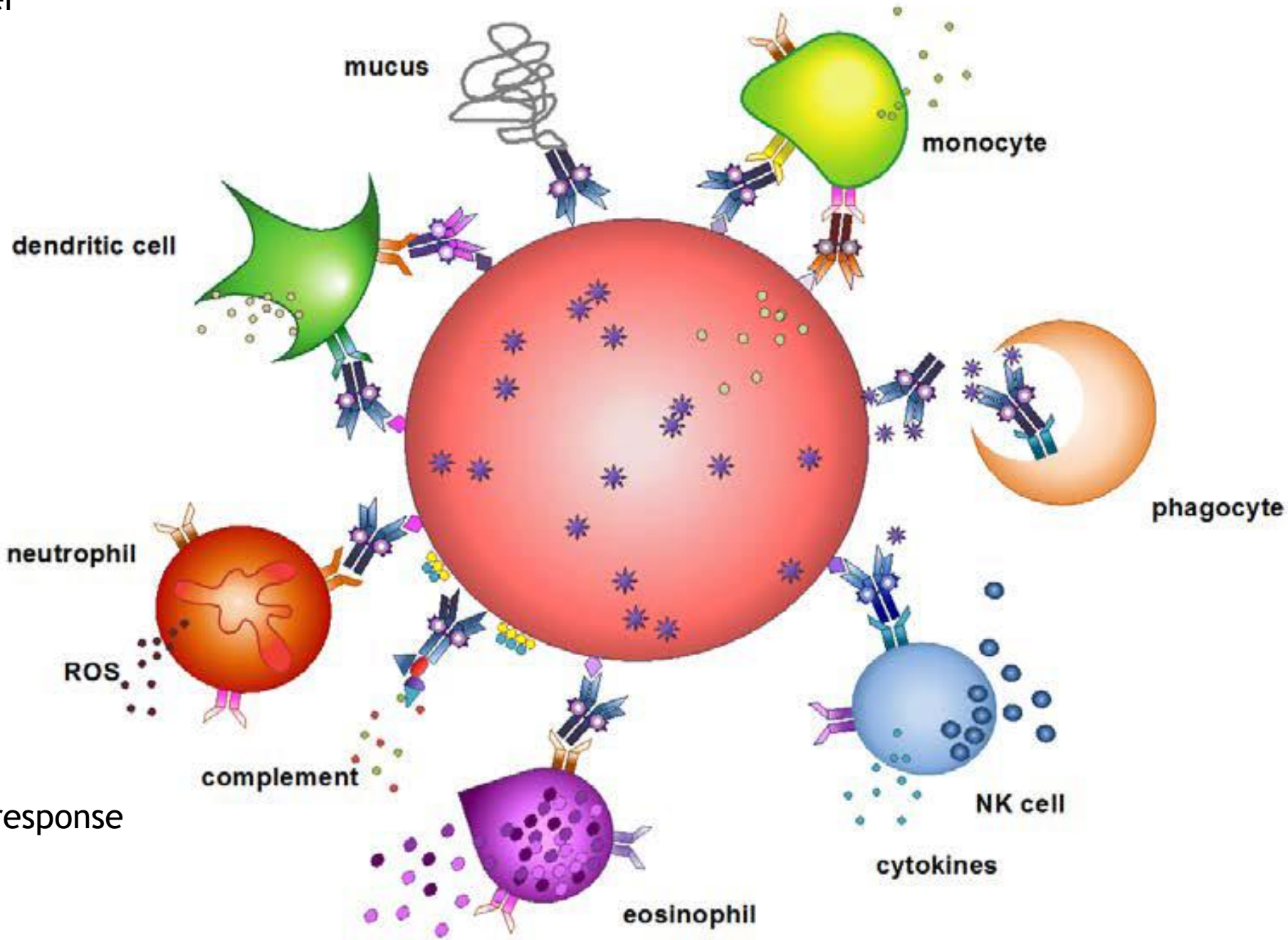


Pneumococcal infection
- Increase risk in asplenia
- T cell deficiency (HIV)
- Antibody deficiency
- Complement deficiency

Relationship with protection?

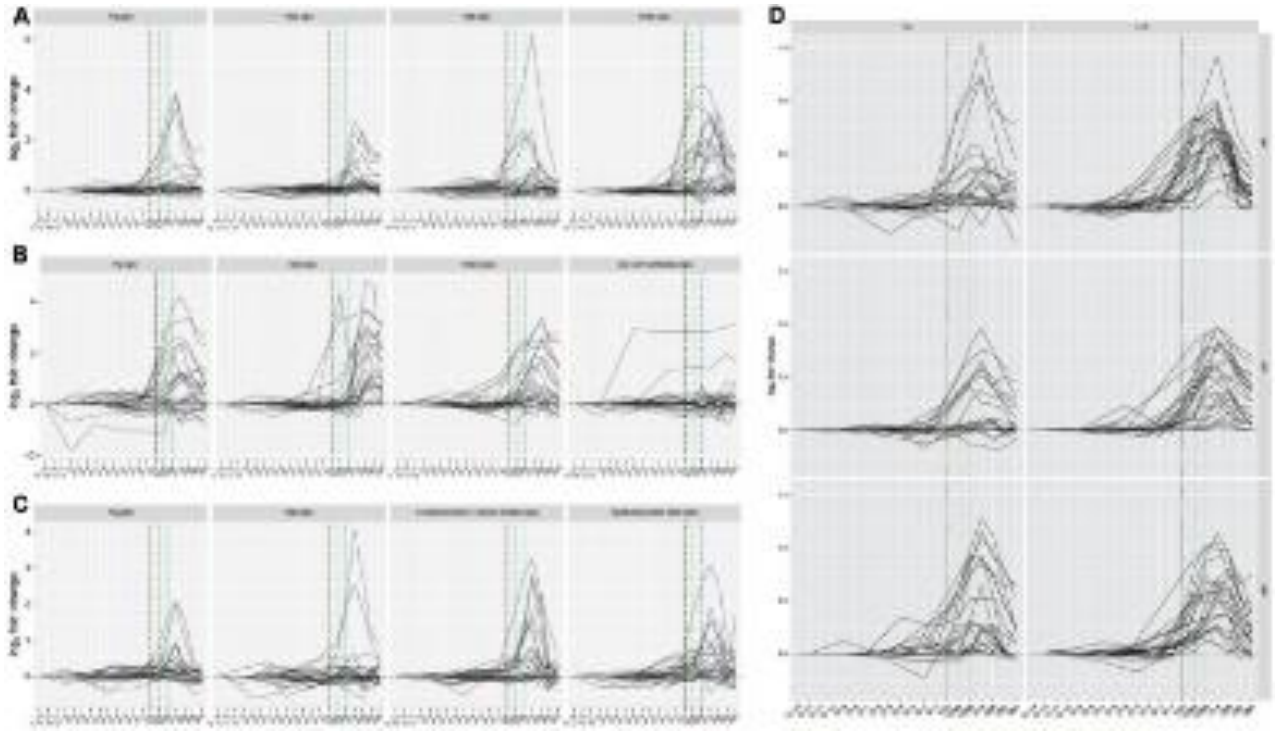


Galit Alter

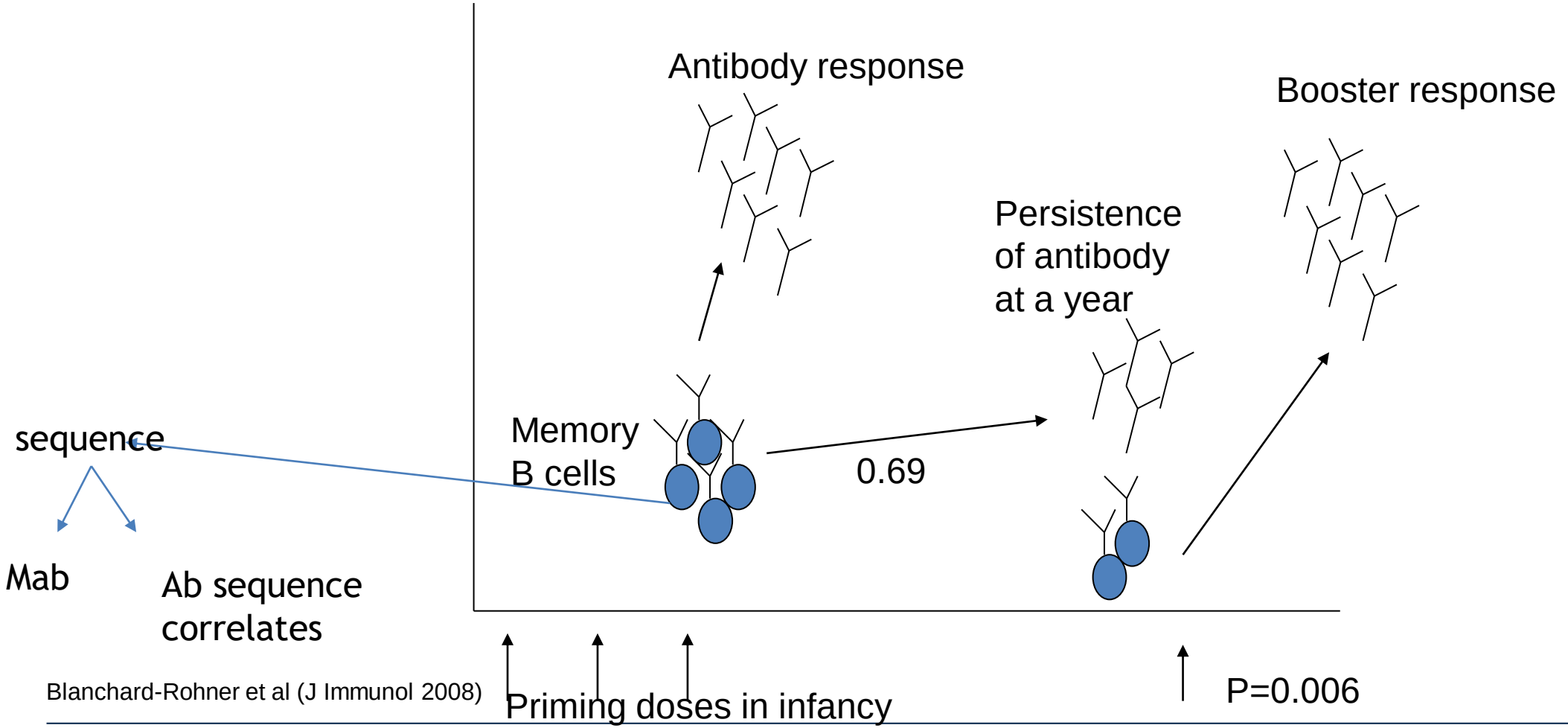


Mucosal response
Avidity
Class
Subclass
Sequence

Bacterial protein array to identify binding antibodies



Magnitude of primary B cell responses may determine persistence of antibody



Blanchard-Rohner et al (J Immunol 2008)

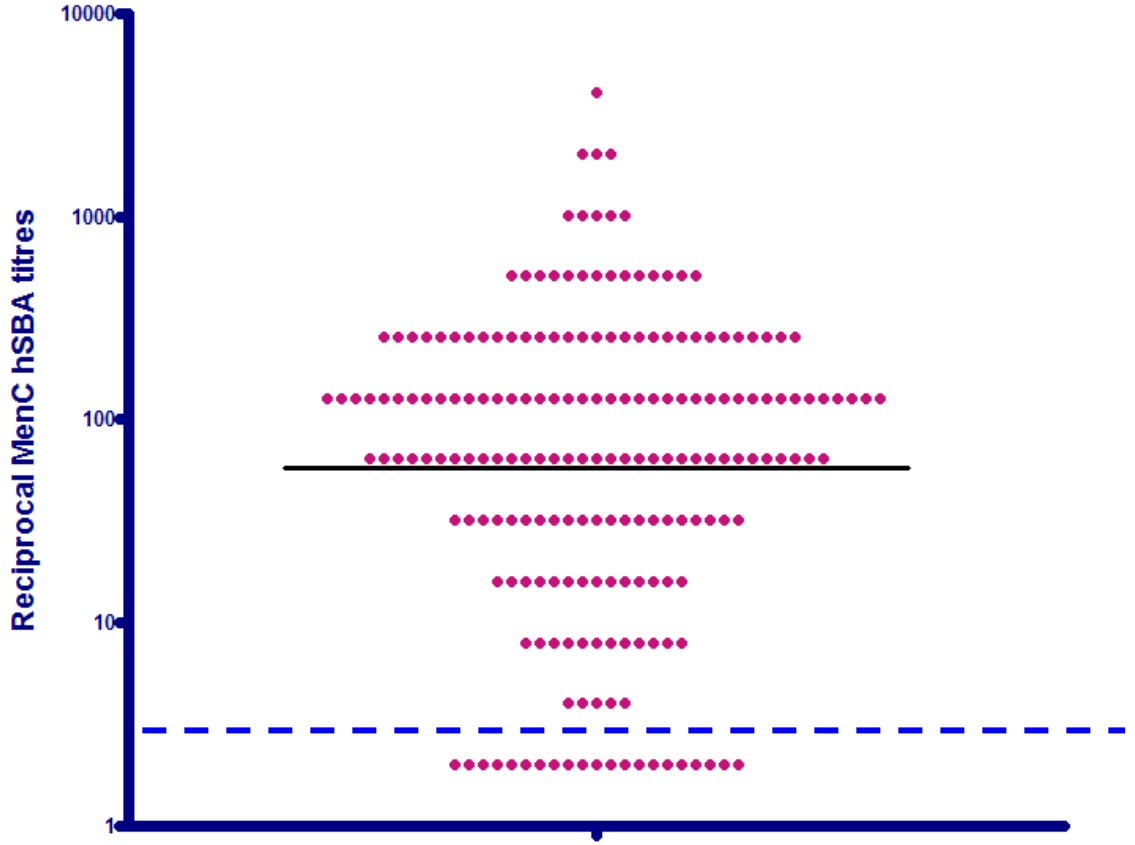
Shared antibody sequences

CDR3 AA sequence	Reference	Length	No. of (related) sequences at baseline	No. of (related) sequences 7 days after vaccination	No. of participants with (related) sequences at baseline	No. of participants with (related) sequences 7 days after vaccination
CARDYFGSGSVYYFDYW	Poulsen 2011	17	0	22	0	3
CARDYFGSGSIYYFDYW	Poulsen 2011	17	0	30	0	3
CASGNTLDYW	Poulsen 2011	10	0	17	0	2
CATGRTLDYW	Poulsen 2011	10	0	35	0	2
CARSVVPATRAFDFW	Poulsen 2011	15	0	2	0	2
CARQTDNWFDPW	Poulsen 2011	12	5	80	2	3
CARDYSSPYYFDYW	Poulsen 2011	14	3	10	2	2
CARDYFGSGPIYYFDHW	Poulsen 2011	17	0	5	0	2
CARDYYGSGSHYYFDYW	Poulsen 2011	17	0	14	0	3
CASGSTLDYW	Poulsen 2011	10	0	21	0	2
CATGNTLDYW	Poulsen 2011	10	0	46	0	2
CTSGVTFDYW	Poulsen 2011	10	2	10	1	2
CARRHYCSSTSCYDAFDIW	Poulsen 2011	19	0	2	0	2
ARHADNWFDP	Frolich 2010	10	1	4	0	3
ARQADNWFDP	Frolich 2010	10	0	71	0	3
AFTADNWFDP	Frolich 2010	10	0	3	0	2
CATGVTLDYW	Dekosky 2013	10	0	34	0	2
CVTGVTLDYW	Dekosky 2013	10	0	10	0	2
CATGFTLDYW	Dekosky 2013	10	0	34	0	2
CATGVTPDYW	Dekosky 2013	10	0	10	0	2
CATGITLDYW	Dekosky 2013	10	0	41	0	2

Known TT-specific CDR3 AA sequences that are found with minor changes in the dataset and are shared by at least 2 study participants

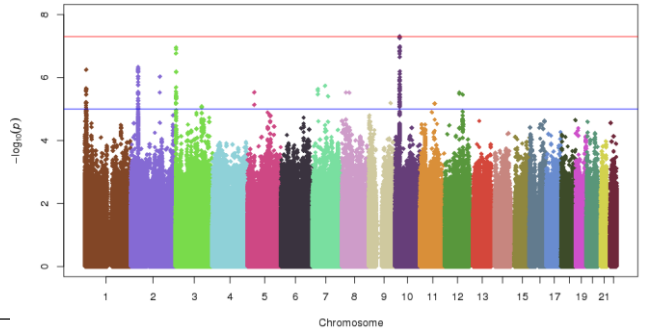
Truck et al 2015

Antibody responses are highly variable



Personalised vaccinology?

Putative level of protection
(Population based)



Twins

Table 1. Key Twin Studies Investigating the Heritability of Vaccine Responses

Reference	Vaccine	Study Population	Response Measured	Heritability
[5]	Inactivated HAV + recombinant HBsAg	192 monozygotic and 190 dizygotic German adult twins aged 18–65 y	Anti-HAV antibody	36%
			Anti-HBsAg antibody	61%
[8]	Pneumococcal vaccination	48 monozygotic and 36 dizygotic Caucasian adult twins aged 21–65 y	IgG and IgG2, serotype-specific antibodies	Yes, varies between serotypes
[7]	Hib conjugate vaccine	86 monozygotic and 294 dizygotic Gambian twins aged 5 mo	Anti-PRP IgG	51%
[6]	Recombinant HBsAg	96 monozygotic and 318 dizygotic Gambian twins aged 5 mo	Anti-HBsAg antibody	77%
[6]	Oral polio vaccine		Anti-poliovirus antibody	60%
[6]	DTP		Anti–tetanus toxoid antibody concentrations	44%
			Anti–diphtheria toxoid antibody concentrations	49%
[6]	BCG, 0.05 mL		Cytokine responses to PPD	46%

Abbreviations: BCG, Bacillus Calmette -Guérin; DTP, diphtheria-tetanus-pertussis; HAV, hepatitis A virus; HBsAg, hepatitis B surface antigen; Hib, *Haemophilus influenzae* type b; IgG, immunoglobulin G; PPD, purified protein derivative; PRP, purified polyribosylribitol phosphate.

O'Connor and Pollard, CID 2013

Non-genetic factors

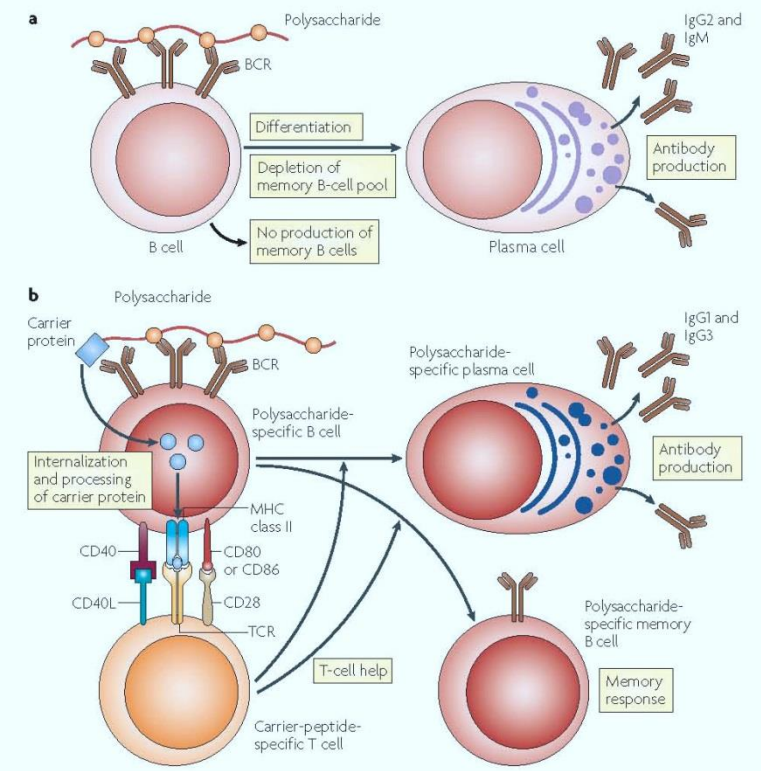
- Maternal antibody
- Nutrition
- Co-morbidity
- Infectious/exposure history
- Drugs
- Microbiome
- Assay performance
- Vaccine type/performance/delivery

Correlates for paediatric vaccines

Antibody correlates	Cell mediated correlates (T cell)
Diphtheria, antibody (toxin neutralisation)	
Tetanus, antibody (toxin neutralisation)	
Hib, antibody (ELISA)	
MenC, antibody (serum bactericidal assay)	
Pneumococcus, antibody (ELISA)	
Hepatitis A, antibody (ELISA)	
Hepatitis B, antibody (ELISA)	
Measles, antibody (microneutralisation)	
Rubella, antibody (immunoprecipitation)	
Varicella, antibody (serum neutralisation or gp ELISA)	
Influenza, antibody (HAI)	
Polio antibody (serum neutralisation)	
Rabies, antibody (serum neutralisation)	

B cell help

Vaccine	T cell involvement?
Diphtheria, tetanus antitoxin, pertussis	B cell help
Hib, MenC, PCV antibody	B cell help
Measles, Rubella, Mumps, Polio, Varicella	B cell help

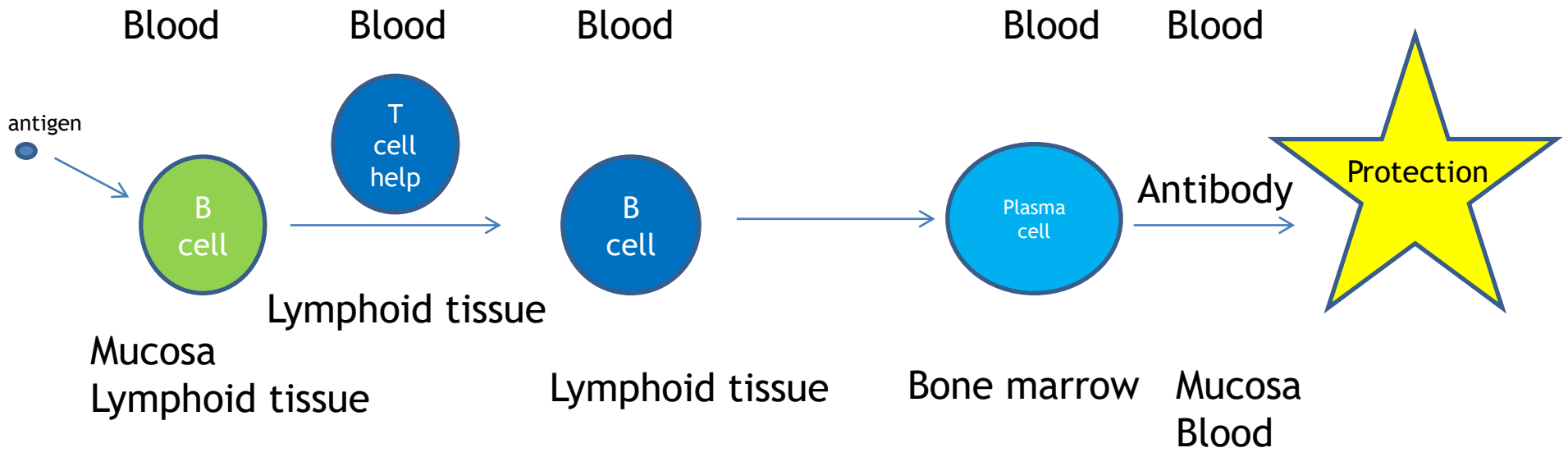


So why don't we have T cell surrogates?

Pollard, Perrett, Beverley. *Nature Rev Immunol.* 2009.

Immune response

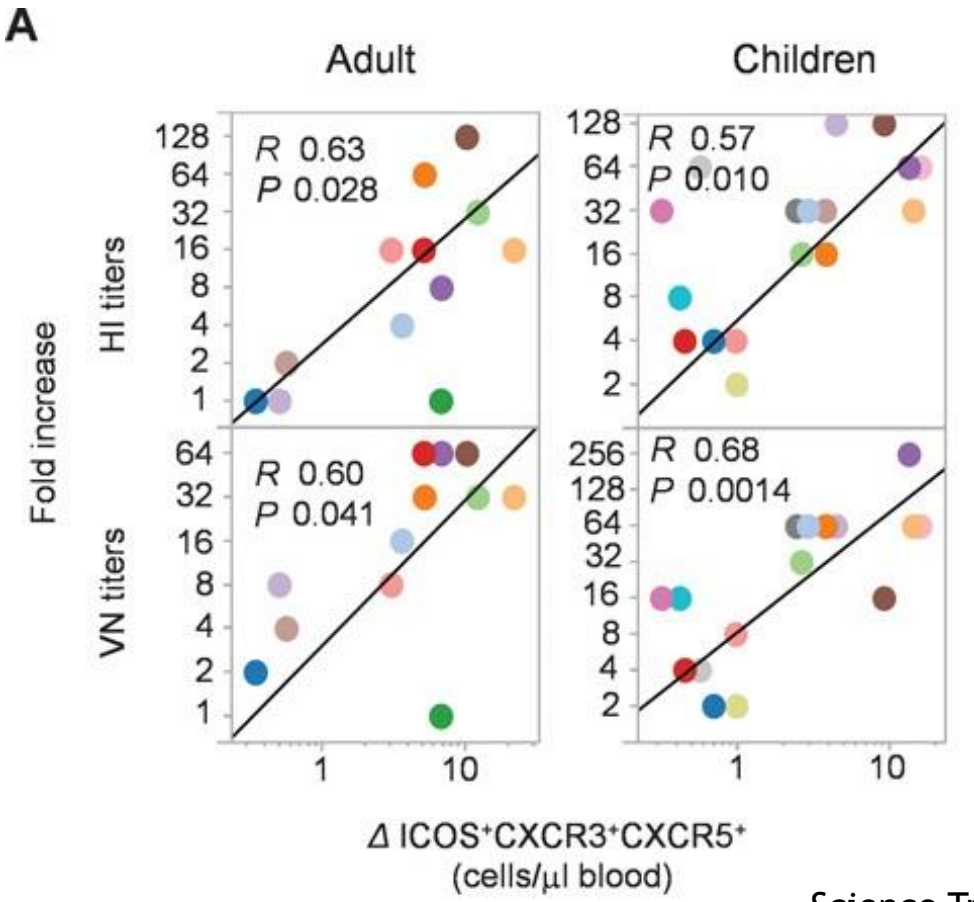
Measurement



Primary location of action

TfH cells correlate with antibody response

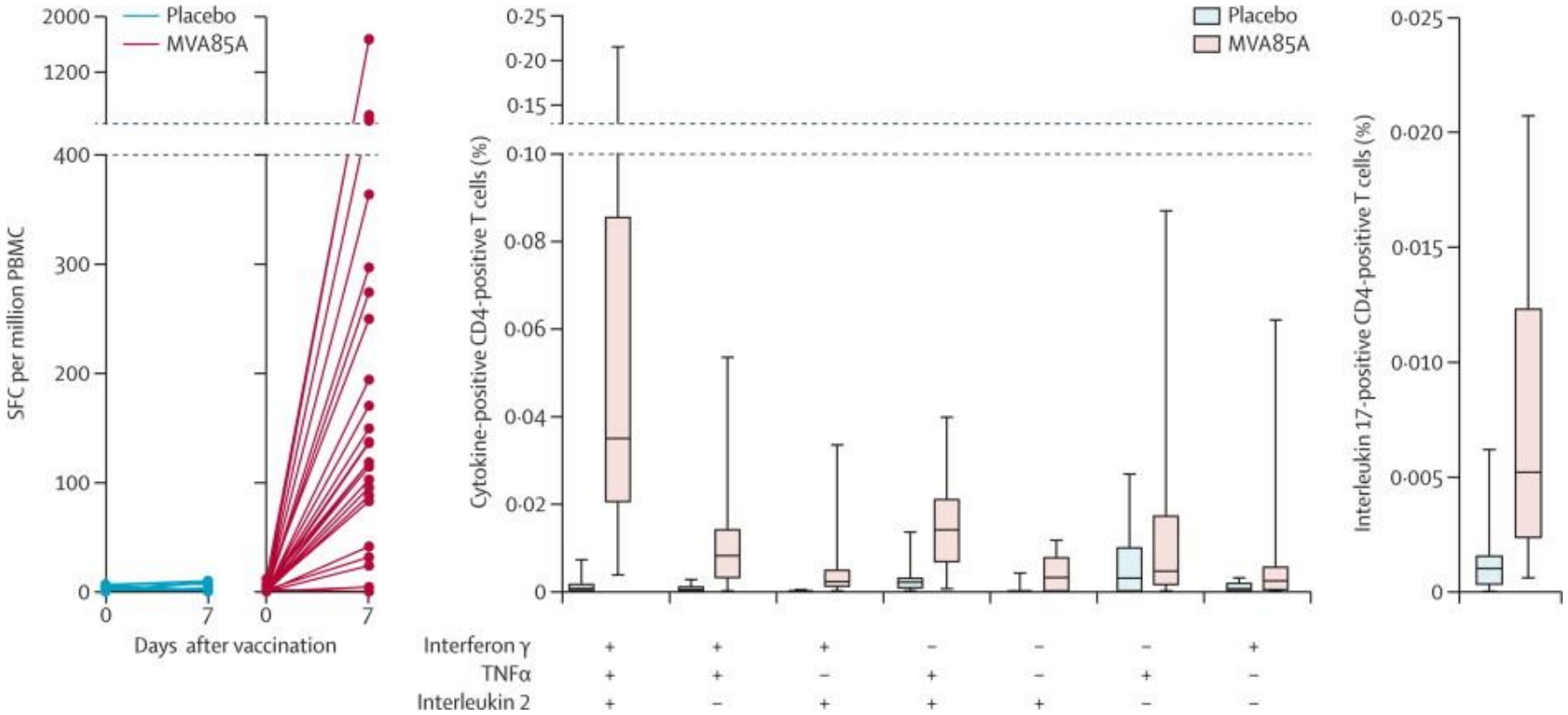
Induction of ICOS+CXCR3+CXCR5+ TH cells correlates with antibody responses to influenza vaccination at 7 days



Science Translational Medicine, 2013

MVA85A

1st infant TB trial since 1968



2797 infants enrolled
BCG or BCG+ MVA85A

Tameris et al, The Lancet 2013

Role of T cells in protection

- Effector T cells limit infection once established
- B cell “help”
- Mucosal role (pneumococcal carriage and Th17)
- Reduce dose of infection?

Age and Immunology

Need multiple doses (? Naïve)
Poor persistence (?bone marrow niche)

"Vaccines don't work"

- Adjuvanted shingles
- Adjuvanted flu
- High dose flu

Time for immunologists to help us understand why these different vaccines work and the rules which should be applied to vaccinology

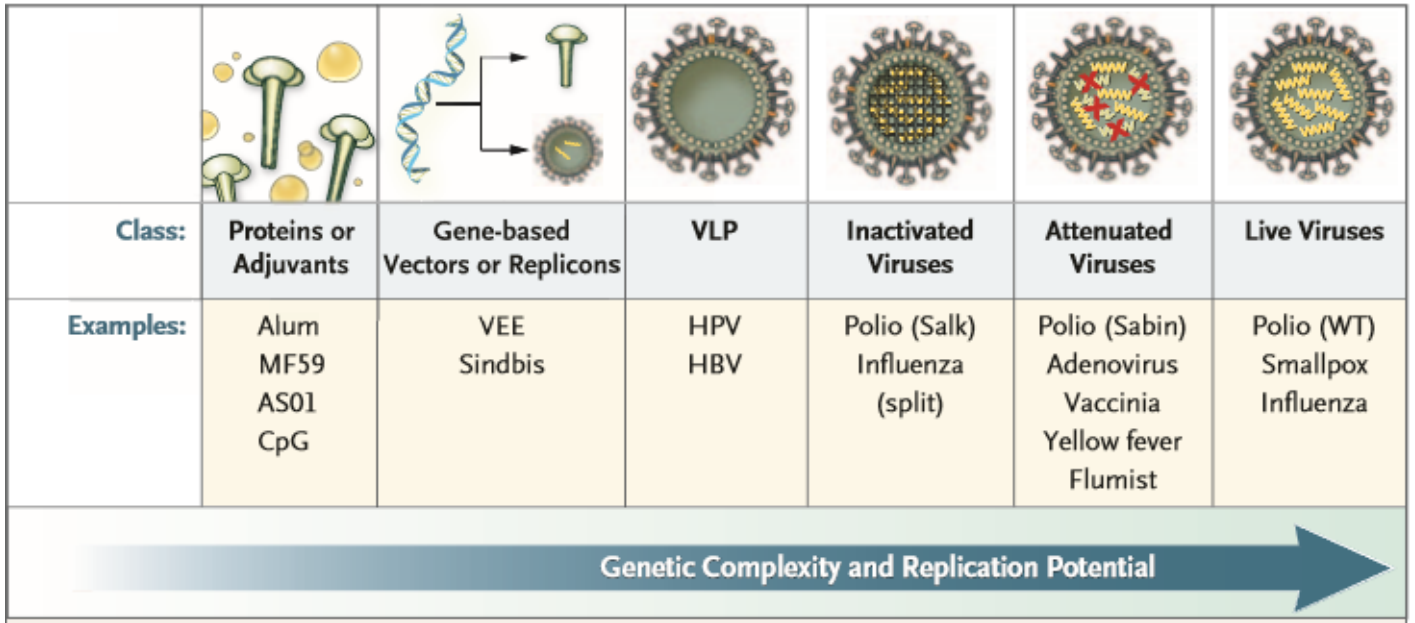


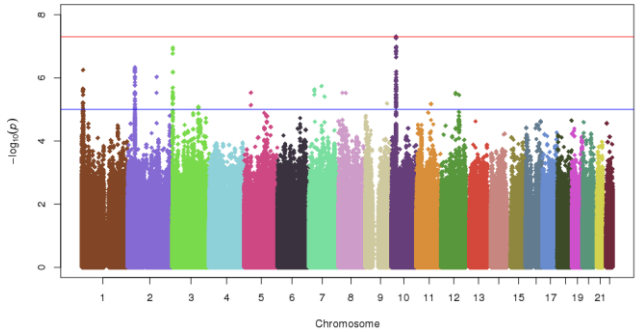
Figure 4. The Spectrum of Costimulation from Adjuvants to Viruses.

A cellular and molecular understanding of dendritic-cell biology has facilitated improvements in vaccine-induced immune responses. Rather than generating responses through infection, immune stimulation can be achieved by increasingly complex modes of antigen presentation that range from introduction of selected proteins, with or without adjuvants, to gene-delivered immunogens, viruslike particles (VLP), structured arrays, or attenuated viruses. These approaches represent a spectrum of complexity and mimicry that elicits protective immunity without inflicting the adverse consequences of natural infection. HBV denotes hepatitis B virus, HPV human papillomavirus, VEE Venezuelan equine encephalitis, and WT wild type.

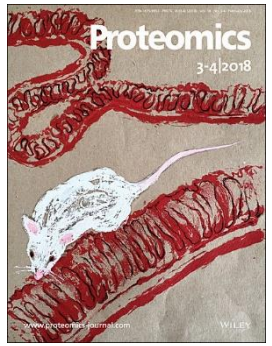
Nabel, 2013 NEJM

Tools

DNA

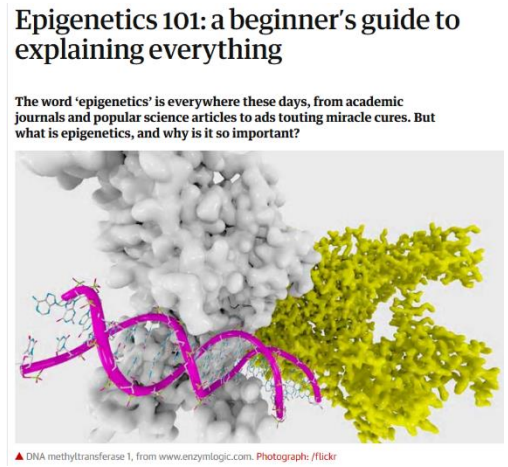


RNAseq

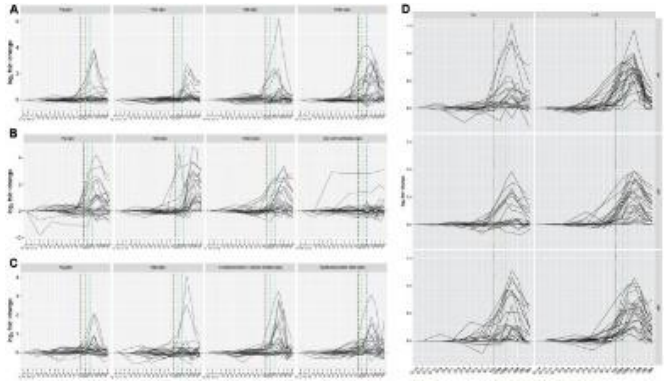


Protein

Cells

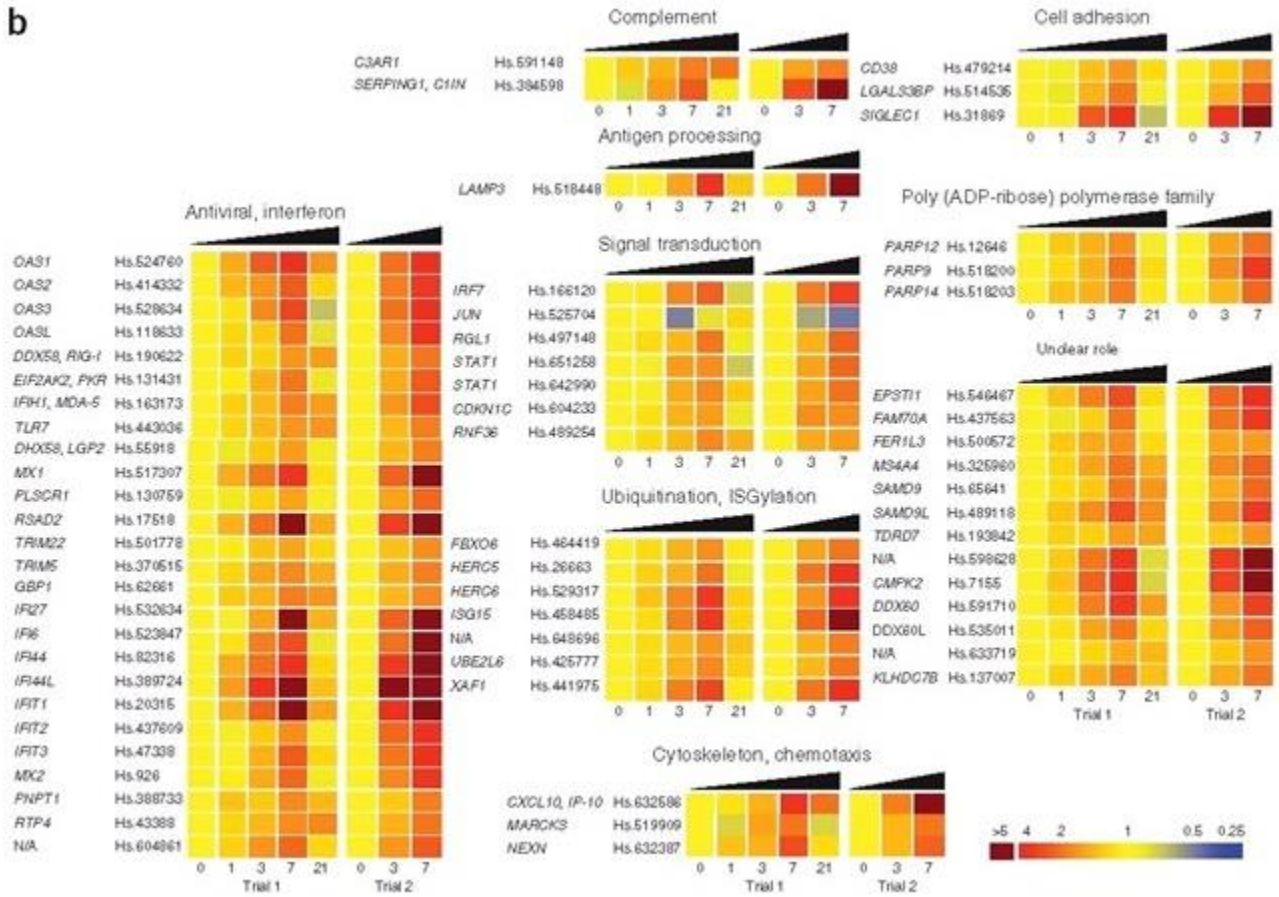


Epigenetics and gene regulation



Antibody

Yellow fever vaccine



Querec et al, Nature Immunology 2009

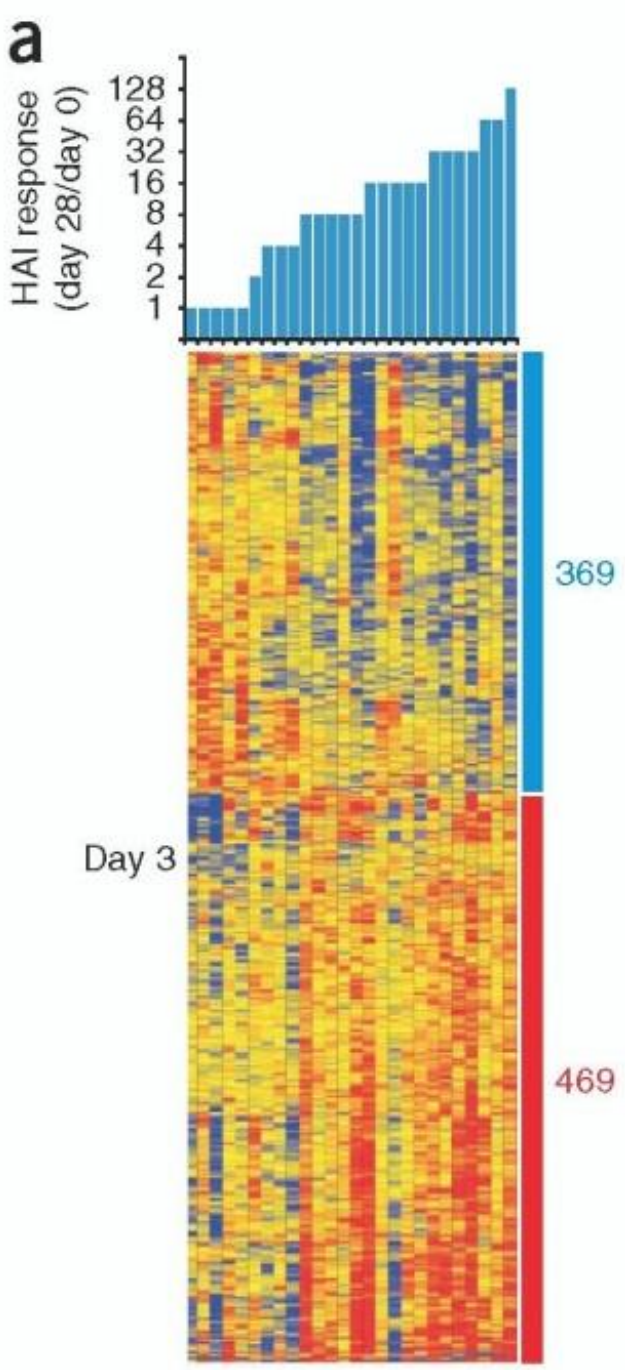
Predicting neutralising antibody titres

Table 5 RT-PCR validation of genes in the DAMIP models for signatures that predict neutralizing antibody titers

Symbol	UniGene	Day	Pearson *r*	*P*-value
BEND4	Hs.120591	7	0.764	0.00002
KBTBD7	Hs.63841	7	0.543	0.02510
TNFRSF17	Hs.2556	7	0.784	0.000001
TPD52	Hs.368433	7	0.530	0.00667

Querec et al, Nature Immunology 2009

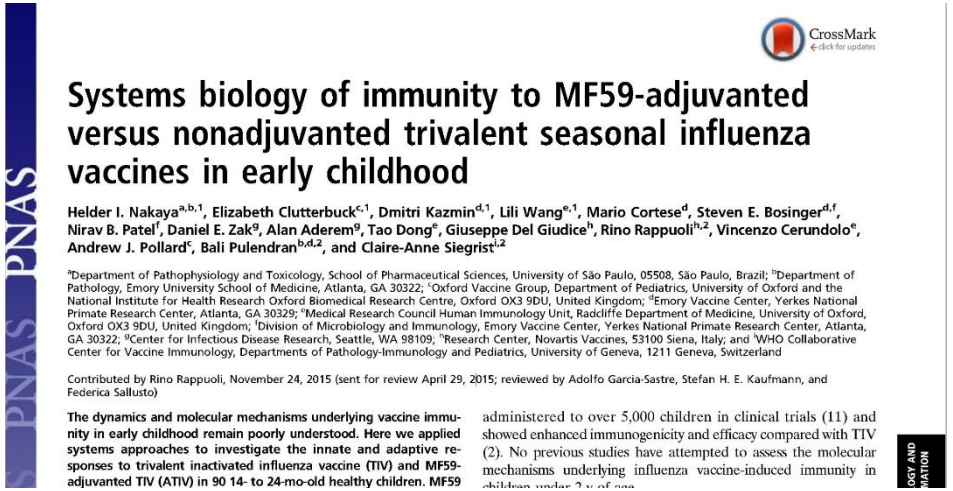
Influenza



Nakaya et al,
Nature
Immunology 2011

Gene expression signatures correlate with influenza antibody (HAI)

Future studies – will gene expression on day 1 be predictive of protection 1 year later?



CrossMark

Systems biology of immunity to MF59-adjuvanted versus nonadjuvanted trivalent seasonal influenza vaccines in early childhood

Helder I. Nakaya[a,b,1], Elizabeth Clutterbuck[c,1], Dmitri Kazmin[d,1], Lili Wang[e,1], Mario Cortese[d], Steven E. Bosinger[d,f], Nirav B. Patel[f], Daniel E. Zak[g], Alan Aderem[g], Tao Dong[e], Giuseppe Del Giudice[h], Rino Rappuoli[h,2], Vincenzo Cerundolo[e], Andrew J. Pollard[c], Bali Pulendran[b,d,2], and Claire-Anne Siegrist[i,2]

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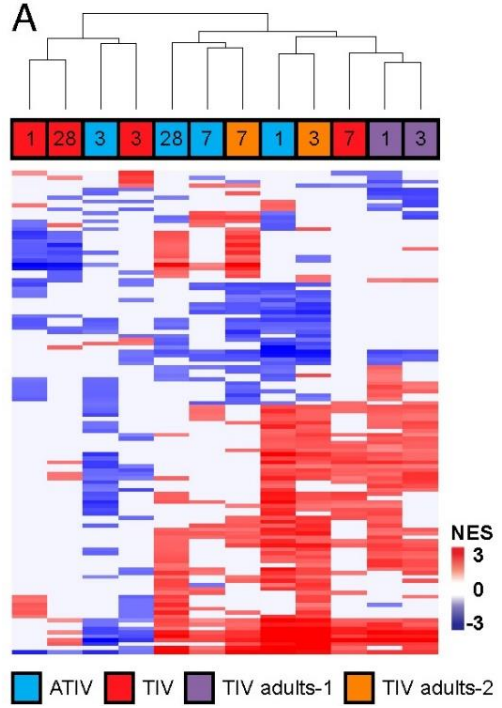
Contributed by Rino Rappuoli, November 24, 2015 (sent for review April 29, 2015; reviewed by Adolfo Garcia-Sastre, Stefan H. E. Kaufmann, and Federica Sallusto)

The dynamics and molecular mechanisms underlying vaccine immunity in early childhood remain poorly understood. Here we applied systems approaches to investigate the innate and adaptive responses to trivalent inactivated influenza vaccine (TIV) and MF59-adjuvanted TIV (ATIV) in 90 14- to 24-mo-old healthy children. MF59

administered to over 5,000 children in clinical trials (11) and showed enhanced immunogenicity and efficacy compared with TIV (2). No previous studies have attempted to assess the molecular mechanisms underlying influenza vaccine-induced immunity in



Characteristics of antigen

Structure-based design

Property analyzed	Techniques	Utility
Three-dimensional structure of antigens and antigen-antibody complexes	X-ray crystallography, NMR, cryo-EM	Allow rational engineering by defining domain boundaries, epitope structure, and underlying architecture
Antigenic structure	ELISA, IP, escape mutant analysis, DXMS, phage display	Define the link between physical structure and the landscapes recognized by antibodies
Post-translational modification	SDS–PAGE, MS, glycosidic linkage analysis, X-ray crystallography, NMR	Assess the authenticity and homogeneity of modifications on recombinantly expressed proteins
Protein folding and stability	CD, ITC, DXMS, NMR, DSC, protease protection, native- and SDS–PAGE	Assess antigen conformation and integrity in solution over time for vaccine stability.
Non-covalent association and hydrodynamic radius	AUC, DLS, SEC, SPR	Assess antigen valency and aggregation

Dormitzer et al, 2008

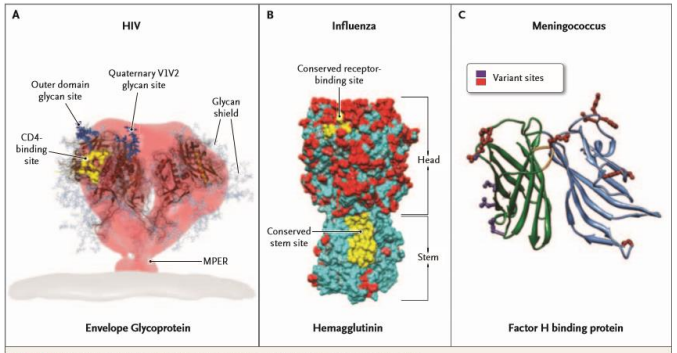
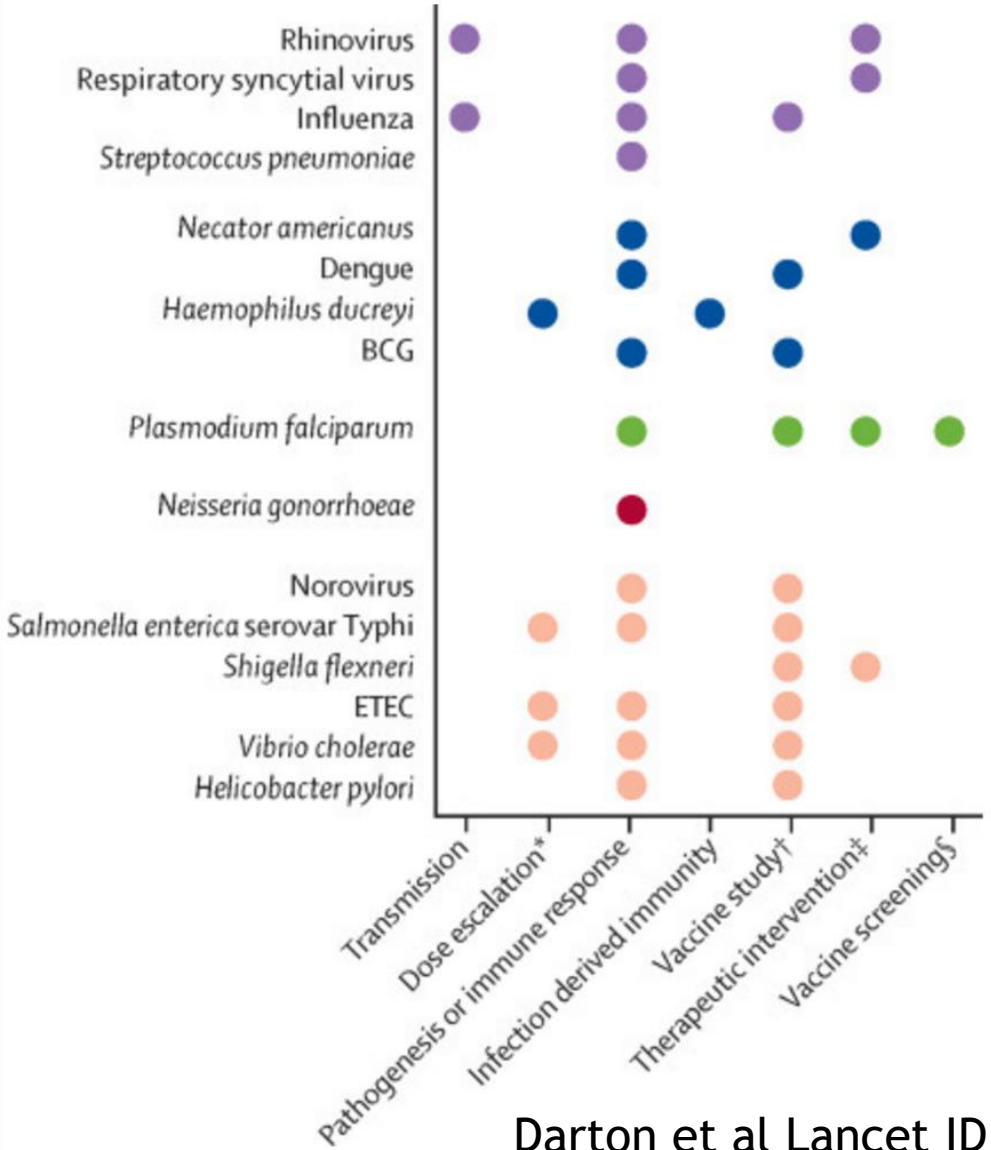


Figure 2. Structure of Viral or Bacterial Glycoproteins and Their Role in Host Invasion.
A detailed knowledge of the mechanism by which viral glycoproteins mediate entry into host cells can now be applied to pathogens that once were not susceptible to vaccines, including human immunodeficiency virus (HIV) (Panel A, Protein Data Bank code 3JWD), influenza virus (Panel B, Protein Data Bank code 1RU7), and meningococcus (Panel C, adapted with permission from Scarselli et al.; Protein Data Bank code 2Y7S).[9-11] MPER denotes membrane proximal external region, and V1V2 variable regions 1 and 2. The Protein Data Bank is accessible at www.pdb.org.

Nabel, 2013 NEJM

Human Challenge studies



Darton et al Lancet ID 2015

Typhoid Challenge model

Clinical trials, industry and data collection

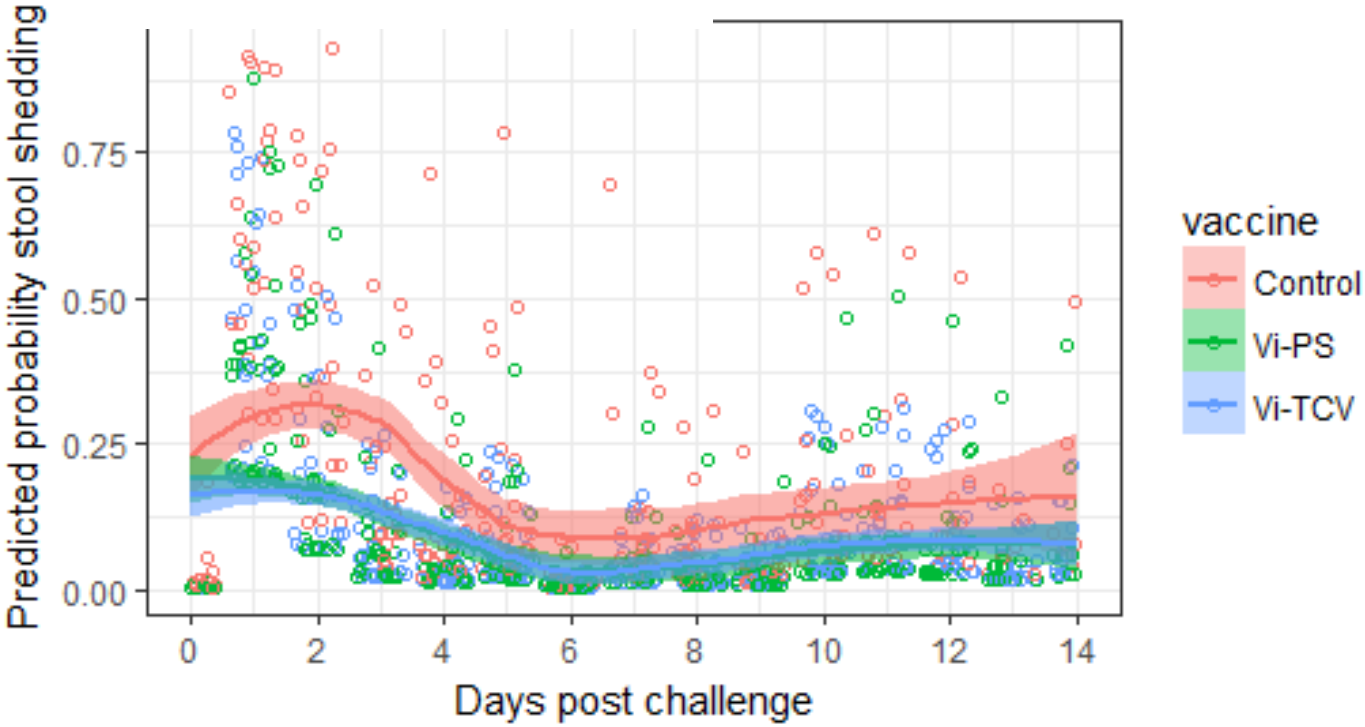
al Research Centre
esearch through partnership

NHS
National Institute for Health Research

Herd immunity?

Odds of shedding overall are 3 times higher if unvaccinated (averaged across all 14 days)

Vaccine	Comparator	OR (95% CI)	P
Control	Vi-PS	3.28 (1.31, 8.19)	0.0111
Control	Vi-TCV	2.88 (1.18, 7.06)	0.0208
Vi-PS	Vi-TCV	0.88 (0.37, 2.11)	0.7729



Conclusions

- Understanding of protective responses can accelerate vaccine development
- Observations from immunodeficiency and natural disease might predict where to look
- Most vaccines have been developed because they made antibody and didn't need any sophisticated immunology
- Antibodies work…….T cells??
- I have ignored the innate immune system but its contribution is now recognised to be far more important than we ever thought

Conclusion

- We have remarkable tools now available that can be used to investigate the immune response but the immune system turns out to be quite complex
- In the future we might harness understanding of the immune response to design vaccines
- Most of the easy vaccines have been done and so vaccinology needs immunology more than ever.