Dissecting the human antibody response to pathogens

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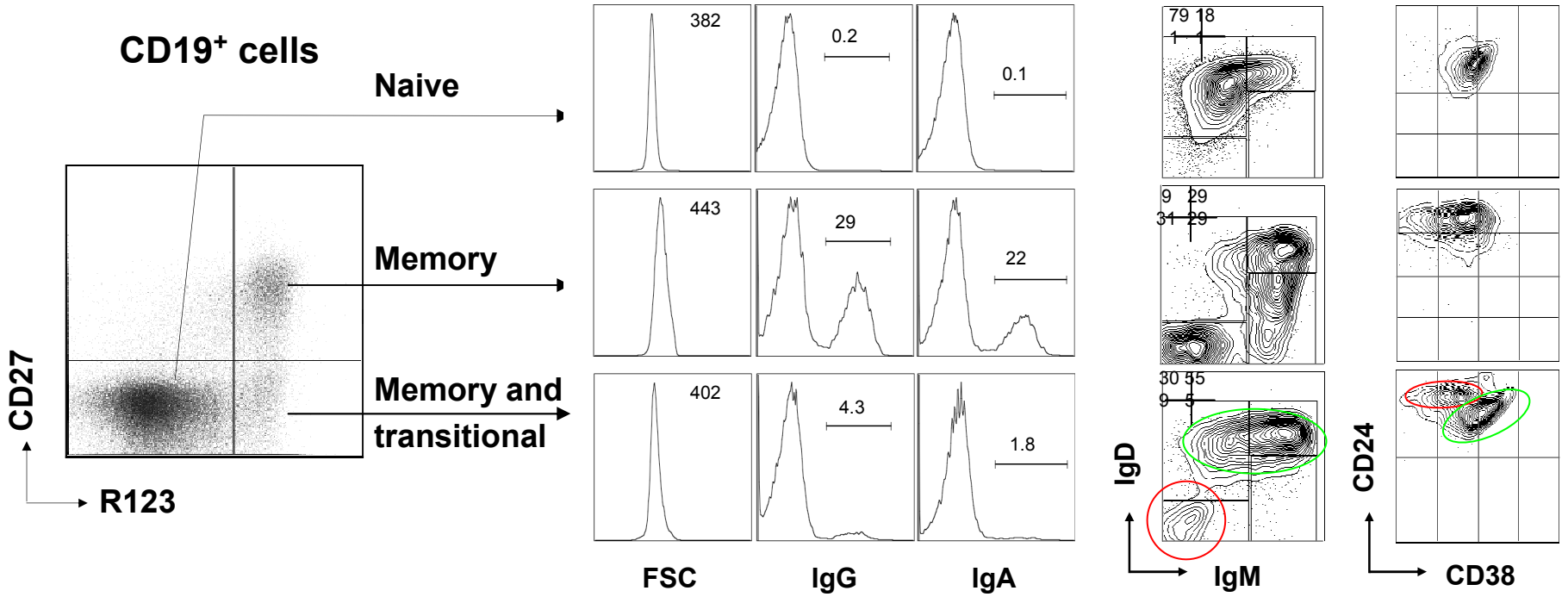
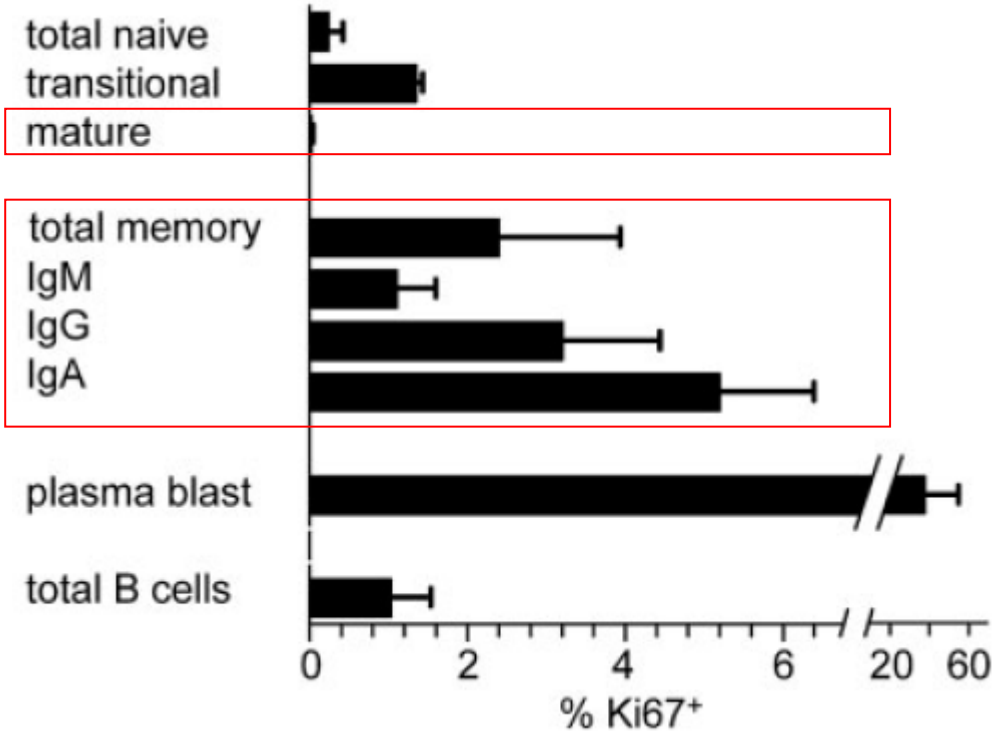
ABCB1 expression precisely identifies human naïve B cells



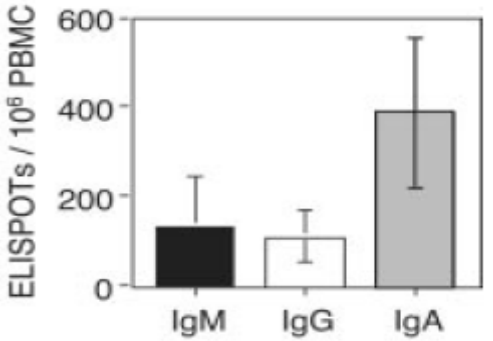
Wirths & Lanzavecchia, *EJI* 2005

High turnover of human memory B cells

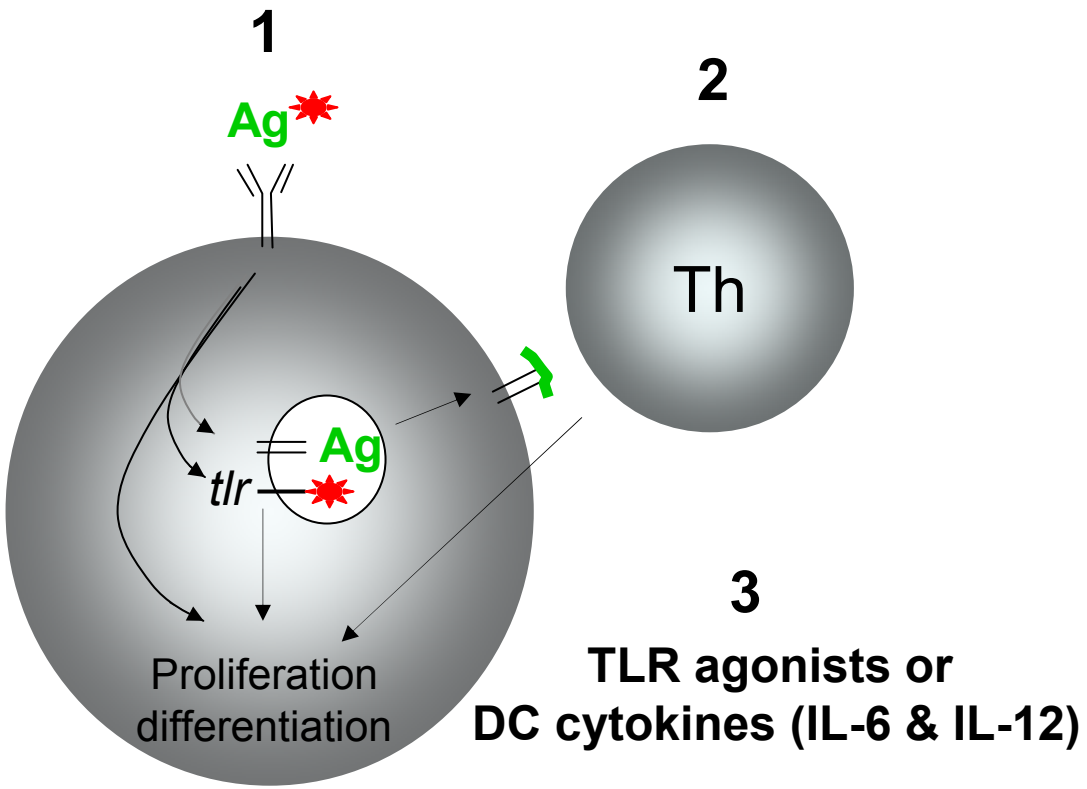
(Ki67 staining)



Plasma blasts



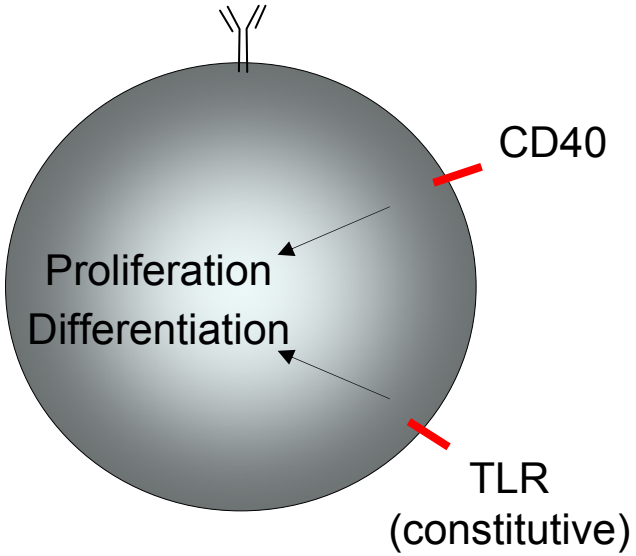
Three signals required for optimal activation of human naïve B cell activation



Human naïve B cells do not express TLRs, but upregulate TLR-2/6/7/9/10 following BCR stimulation

Bernasconi et al, Blood 2002
Ruprecht et al. EJI 2006

Human memory B cells respond to polyclonal stimuli



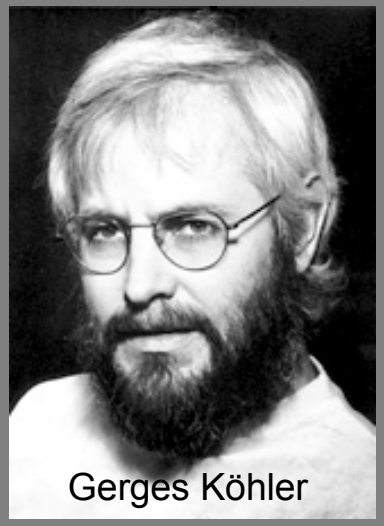
- Bystander T cell help
- Cytokines (IL-15)
- TLR agonists

Bernasconi et al, Science 2002

From serotherapy to monoclonal antibodies

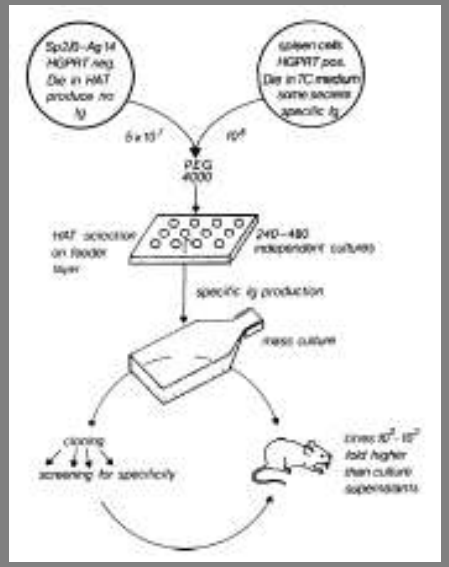
Emil von Behring

Shibasaburo Kitasato

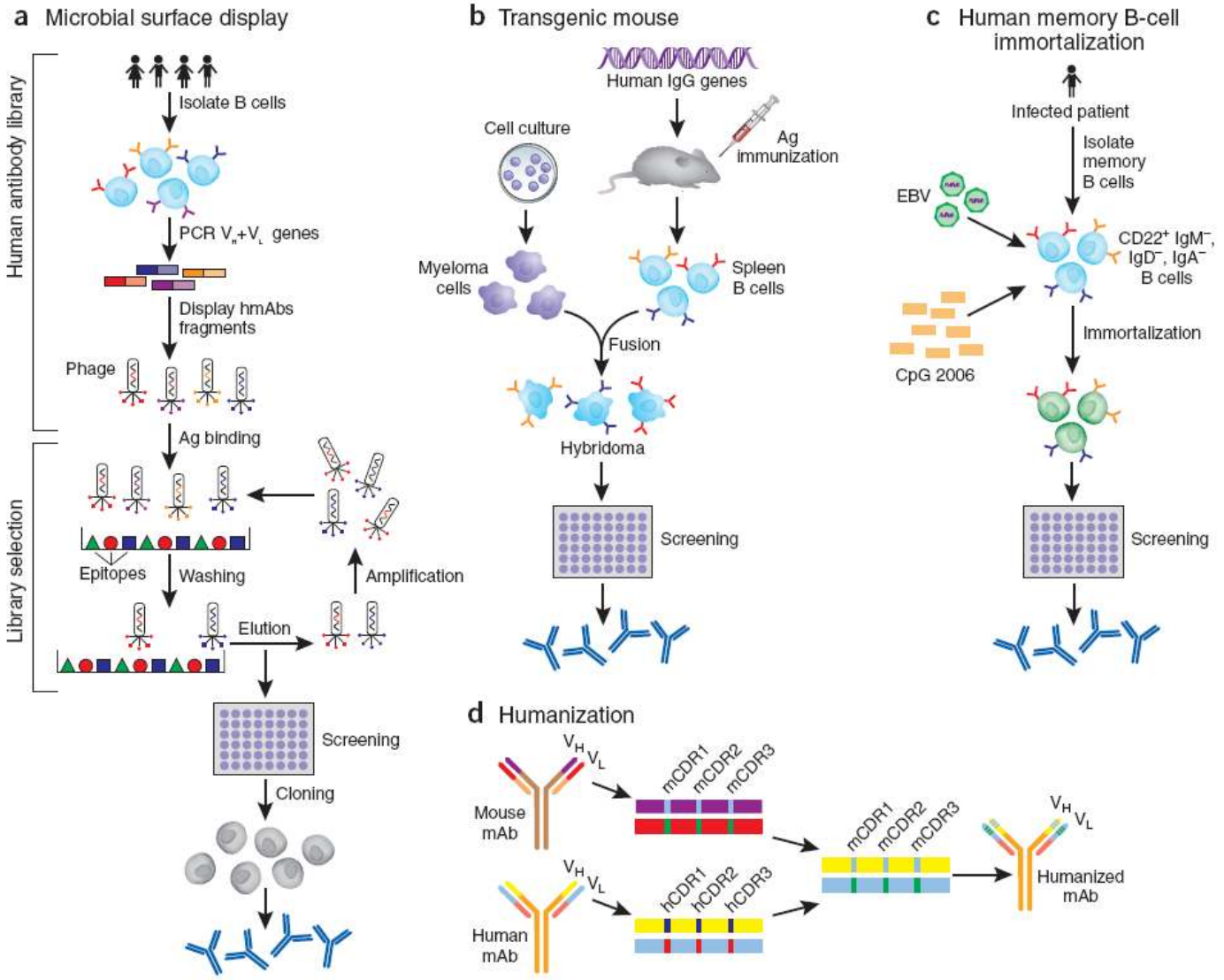


Gerges Köhler

Cesar Milstein



Human antibody techniques



Marasco, Nature Biotech. 2007

The advantage of the human system

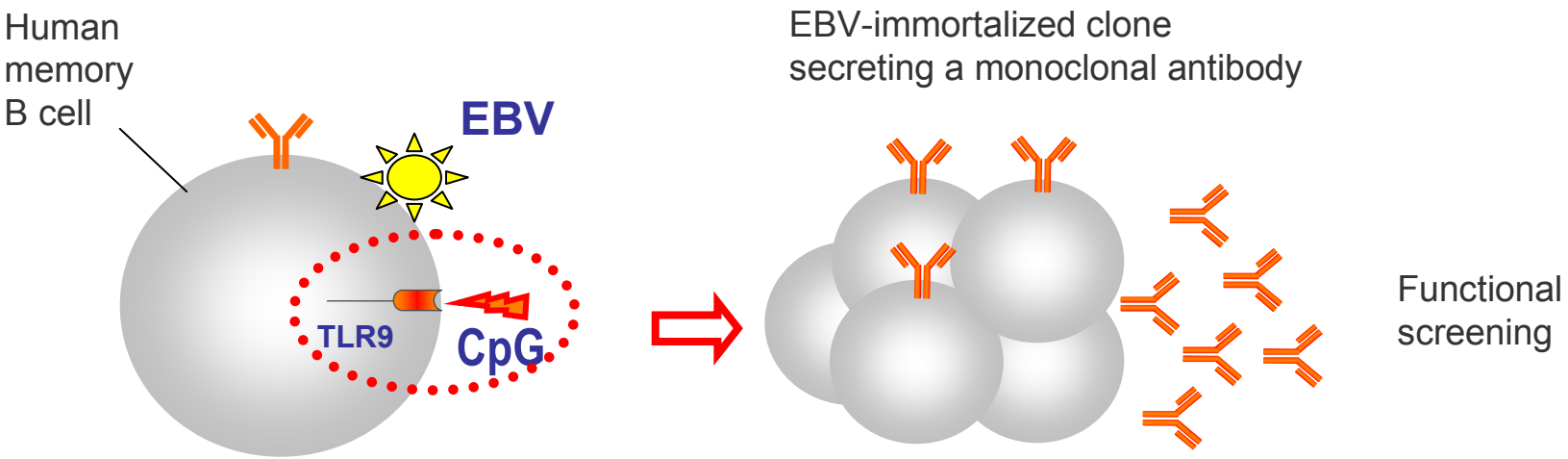
- Large repertoire (10^{10} lymphocytes)
- Large outbred population (high responders)
- Sustained and repeated stimulations
- Response to replicating human pathogens
- Memory B cells are maintained for a lifetime
- mAbs are human and express well in mammalian cells

Sources of human monoclonal antibodies

- Mice with human Ig loci
- Random human Ig libraries
- **Human immmune Ig libraries**
- **Human memory B cells**
- **Human plasma cells**

Single cell PCR versus culture methods

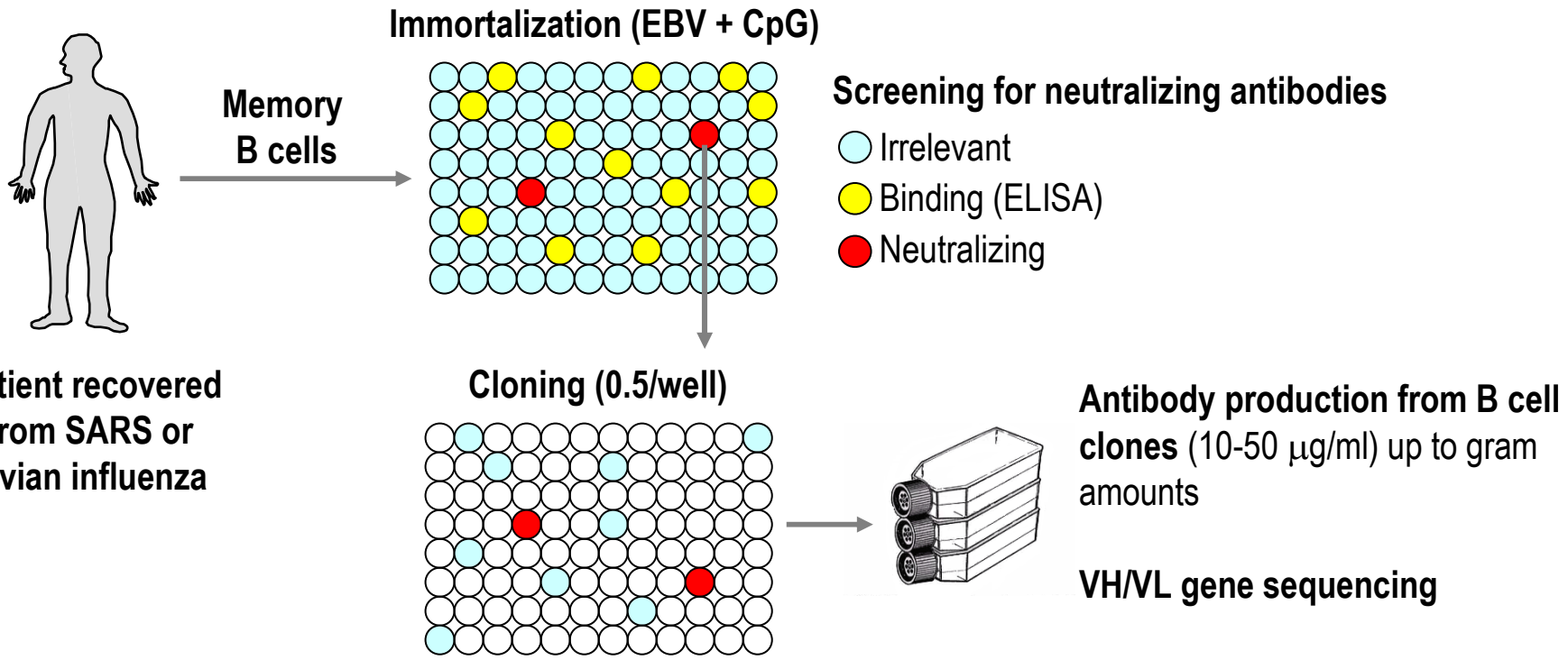
(1) Immortalization of human memory B cells



- **TLR agonists (e.g. CpG) increase by more than 100-fold the efficiency of B cell immortalization by Epstein Barr Virus**
- **The cloning efficiency of memory B cells ranges from 20 to 40%**

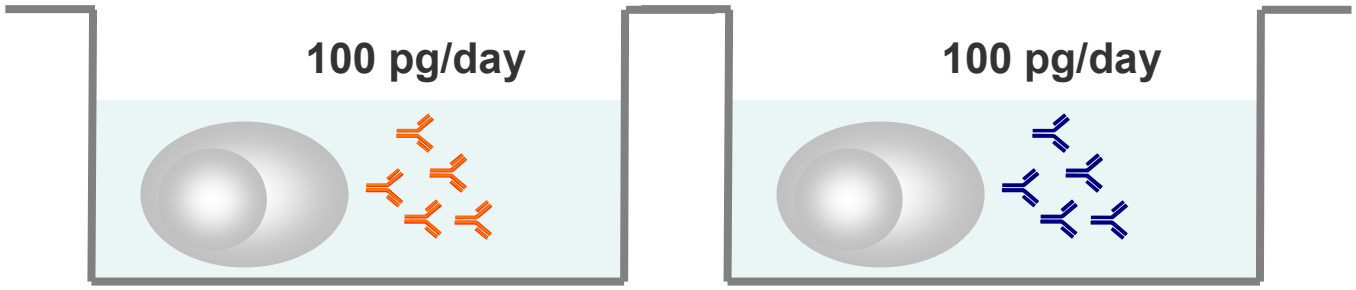
Traggiai et al. *Nature Medicine* 2004

Proof of principle: potent and broadly neutralizing antibodies to SARS-CoV and H5N1



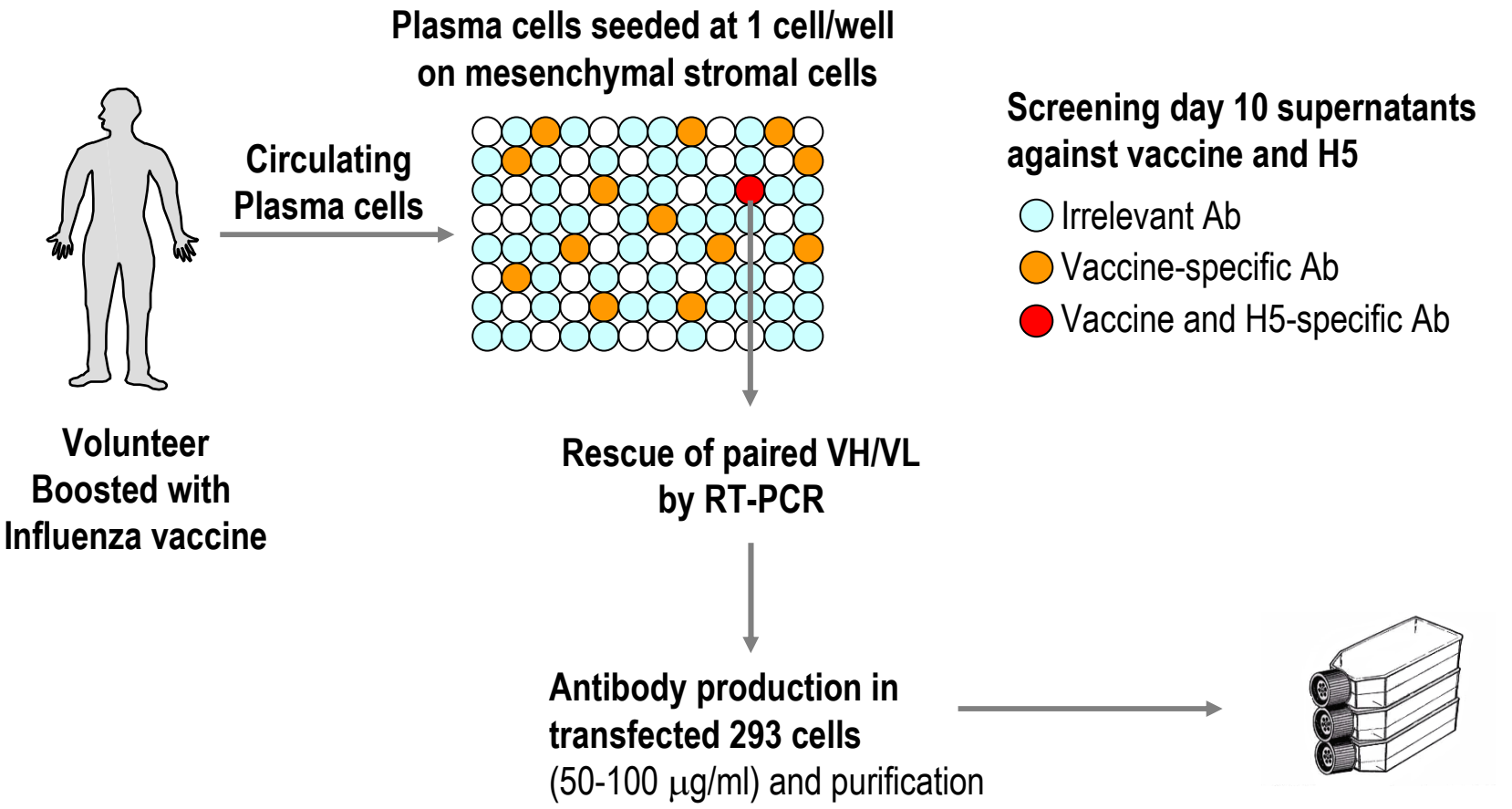
Traggiai et al. *Nature Medicine* 2004
Simmons et al *PLoS Medicine*, 2007

(2) Long term culture of human plasma cells



- Individual plasma cells are cultured on stromal cells so that the secreted antibody accumulates in the culture supernatant
- Screening can be performed using multiple parallel assays
- Once a cell is identified, the VH/VL genes are rescued by single cell RT-PCR and the antibody is produced in a recombinant form

Proof of principle: antibodies that neutralize multiple influenza virus subtypes



Examples

Dengue

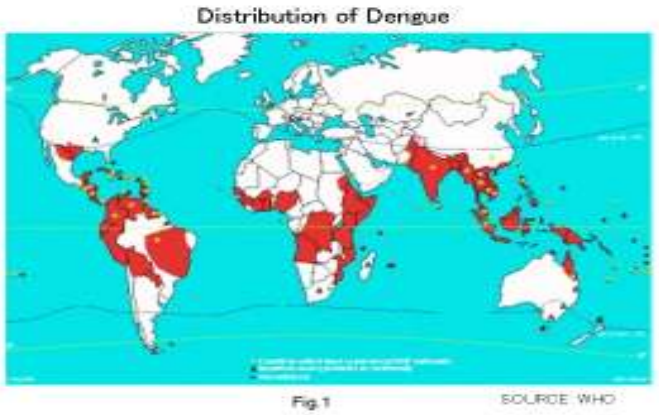
HIV-1

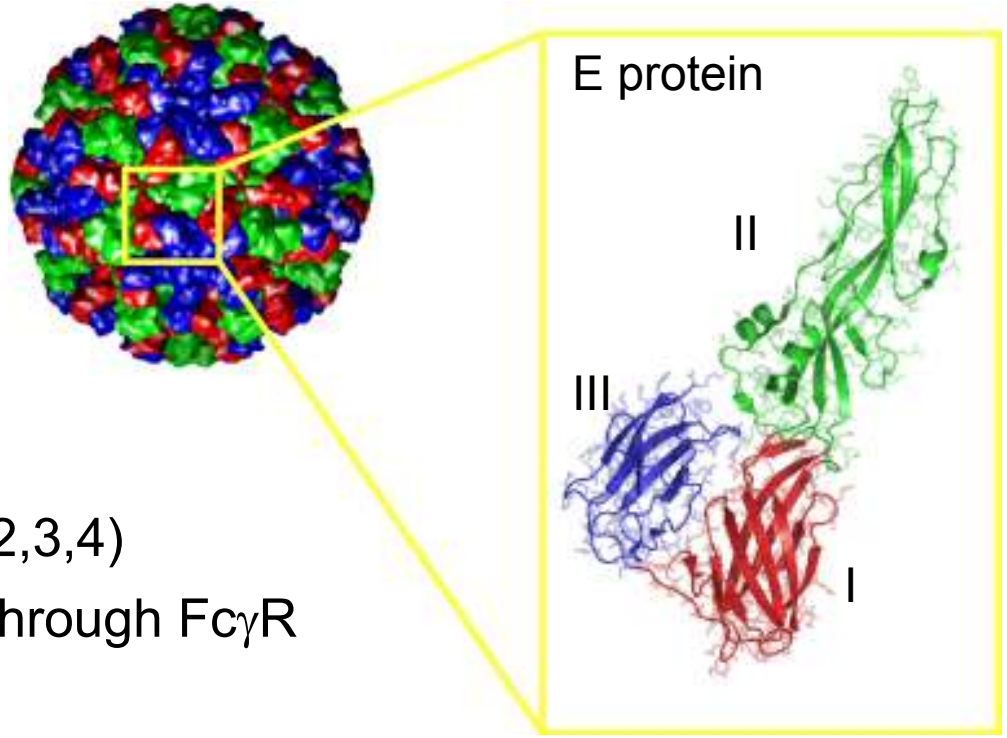
Influenza

HCMV

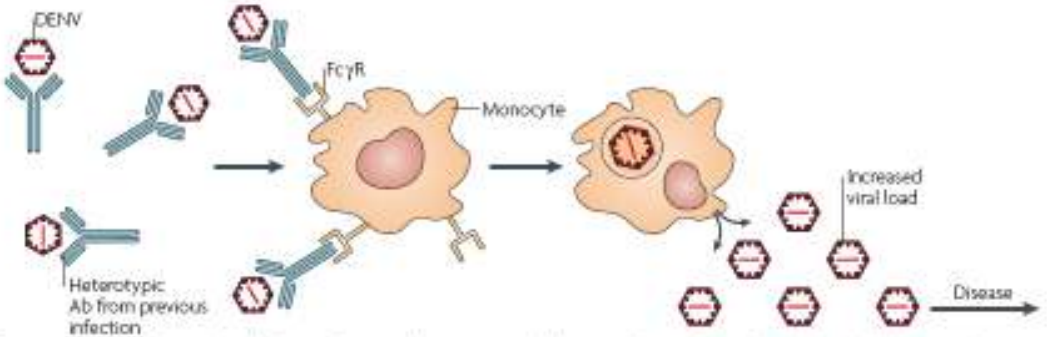
Malaria

Dengue virus: neutralization versus enhancement

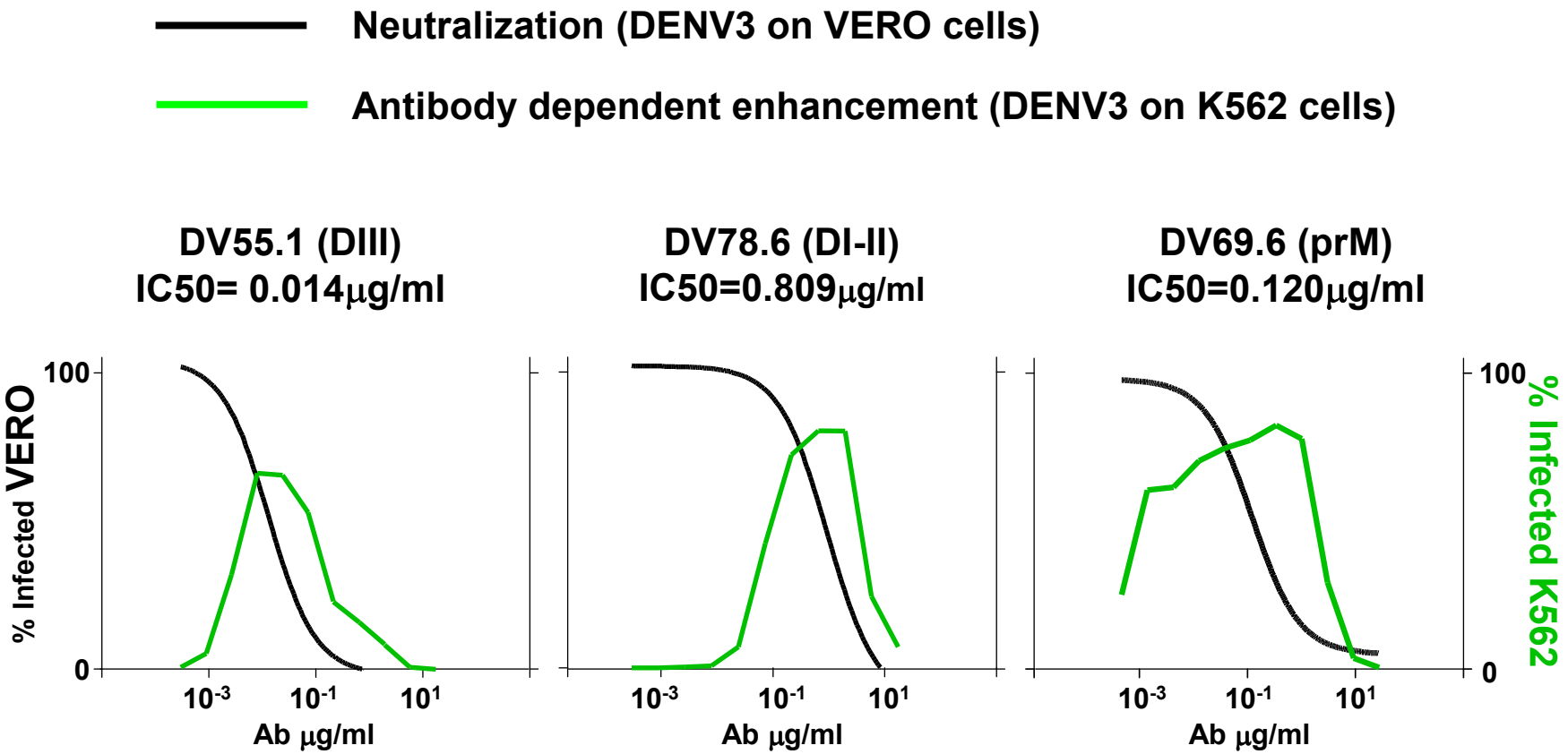




- Four major serotypes (DEN1,2,3,4)
- Abs can enhance virus entry through FcγR



Neutralization and enhancement of DENV infection by Human Monoclonal Antibodies



Specificity of DENV neutralizing human mAbs

Primary DENV3 infection

		ICS C6/36				Neutralization (IC_{50})				Enhancement (range log µg/ml)			
h-mAbs	Specificity	DV1	DV2	DV3	DV4	DV1	DV2	DV3	DV4	DV1	DV2	DV3	DV4
DV 55.1	E, DIII	+	+	+	—	+++	+	+++	—	-3 , 0	-1 , 1	-3, 0	—
DV 63.1	E, DIII	+	—	+	—	+++	—	+++	—	-4 , -1	—	-3 , 0	—
DV 78.6	E, DI-II	+	+	+	+	+	+	+	+	-2 , 1	-2 , 1	-2 , 1	-2 , 1
DV 82.11	E, DI-II	+	+	+	+	++	++	++	+	-2 , 1	-2 , 1	-2 , 1	-2 , 1
DV 90.3	E, DI-II	+	—	+	—	+	—	+	—	-2 , 1	—	-1 , 1	—
DV 79.3	E, DI-II	—	—	+	—	—	—	++	—	—	—	-2 , 0	—
DV 64.3	prM	+	+	+	+	+	+	++	++	-4 , 1	-4 , 1	-4 , 1	-3 , 1
DV 69.6	prM	+	+	+	+	+	+	++	++	-3 , 1	-3 , 1	-3 , 1	-3, 2
DV 57.4	E	+	+	+	+	—	+	+	+	—	-3, 0	-3, 0	-2 , 0
DV 66.1	E	+	+	+	+	—	—	±	—	—	—	-2 , 1	—
DV 52.1	prM	+	+	+	+	—	—	—	—	—	—	-3 , 1	-1 , 1
DV 61.2	E	+	+	+	+	—	—	—	—	0 , 2	-2 , 1	-2 , 1	-2 , 1
DV 75.9	prM	+	+	+	—	—	—	—	—	-2 , 0	-1 , 2	-3, 0	-2 , 0
DV 76.5	E,	+	+	+	+	—	—	—	—	-1 , 2	-2 , 1	-2 , 1	-2 , 1
DV 77.5	E	+	+	+	+	—	—	—	—	-1 , 2	-2 , 0	-2 , 1	-2 , 1
DV 62.5		+	+	+	+	—	—	—	—	-2 , 1	-2 , 1	-2 , 1	-2 , 1
DV 58.1		+	—	+	+	—	—	—	—	—	—	—	—
DV 86.2	prM	+	+	+	+	—	—	—	—	—	—	—	—

< 0.020 µg/ml
0.021-0.150 µg/ml
> 0.150 µg/ml

< 2 log
3 log
>4 log

Specificity of DENV neutralizing h-mAbs

Secondary infection

		ICS C6/36				Neutralization (IC_{50} µg/ml)				Enhancement (range log µg/ml)			
h-mAbs	Specificity	DV1	DV2	DV3	DV4	DV1	DV2	DV3	DV4	DV1	DV2	DV3	DV4
DV 1.1	E, DI-II	+	+	+	+	+	++	+	++	-2 , 1	-2 , 1	-2 , 1	-2 , 1
DV 4.4	E, DI-II	+	+	+	+	+	+	+	+	-2 , 0	-2 , 1	-1 , 1	-2 , 1
DV 5.1	E, DI-II	+	+	+	+	+	++	+	++	-2 , 1	-2 , 1	-2 , 1	-3 , 1
DV 6.1	E, DI-II	+	+	+	+	+	+	+	+	-1 , 1	-2 , 1	-1 , 1	-2 , 1
DV 7.5	E, DI-II	+	+	+	+	+	++	++	++	-2 , 1	-2 , 1	-2 , 1	-2 , 1
DV 8.1	E, DI-II	+	+	+	+	++	++	++	+++	-3 , 2	-3 , 1	-3 , 1	-3 , 1
DV 13.4	E, DI-II	+	+	+	+	++	+++	+++	++	-2 , 1	-3 , 0	-3 , 0	-3 , 0
DV 14.5	E, DI-II	+	+	+	+	++	++	++	++	-2 , 0	-2 , 0	-2 , 0	-3 , 0
DV 15.7	E, DI-II	+	+	+	+	++	++	+	++	-2 , 1	-3 , 1	-2 , 0	-3 , 1
DV 16.5	E, DI-II	+	+	+	+	+	++	+	++	-2 , 1	-2 , 1	-2 , 1	-2 , 1
DV 17.6	E, DI-II	+	+	+	+	+	++	+	++	-2 , 1	-2 , 1	-2 , 1	-2 , 2
DV 19.3	E, DI-II	+	+	+	+	+	+	+	++	-2 , 1	-2 , 1	-2 , 1	-2 , 1
DV 20.1	E, DI-II	+	+	+	+	+	++	+	+	-2 , 1	-2 , 1	-1 , 1	-2 , 1
DV 21.1	E, DI-II	+	+	+	+	+	++	++	++	-2 , 1	-2 , 1	-3 , 1	-3 , 1
DV 28.8	E, DI-II	+	+	+	+	++	++	+	++	-2 , 0	-2 , 0	-2 , 0	-2 , 1
DV 38.1	E, DI-II	+	+	+	+	+	++	+	++	-2 , 1	-2 , 1	-2 , 1	-3 , 1
DV 27.2		+	+	+	+	+	+	++	++	-3 , 1	-3 , 1	-3 , 1	-3 , 1
DV 34.3		—	+	—	+	—	+++	—	+	—	-3 , 0	—	-1 , 2
DV 3.1		+	+	+	+	—	—	—	—	—	—	—	—
DV 18.6		+	+	+	+	—	—	—	—	—	—	—	—

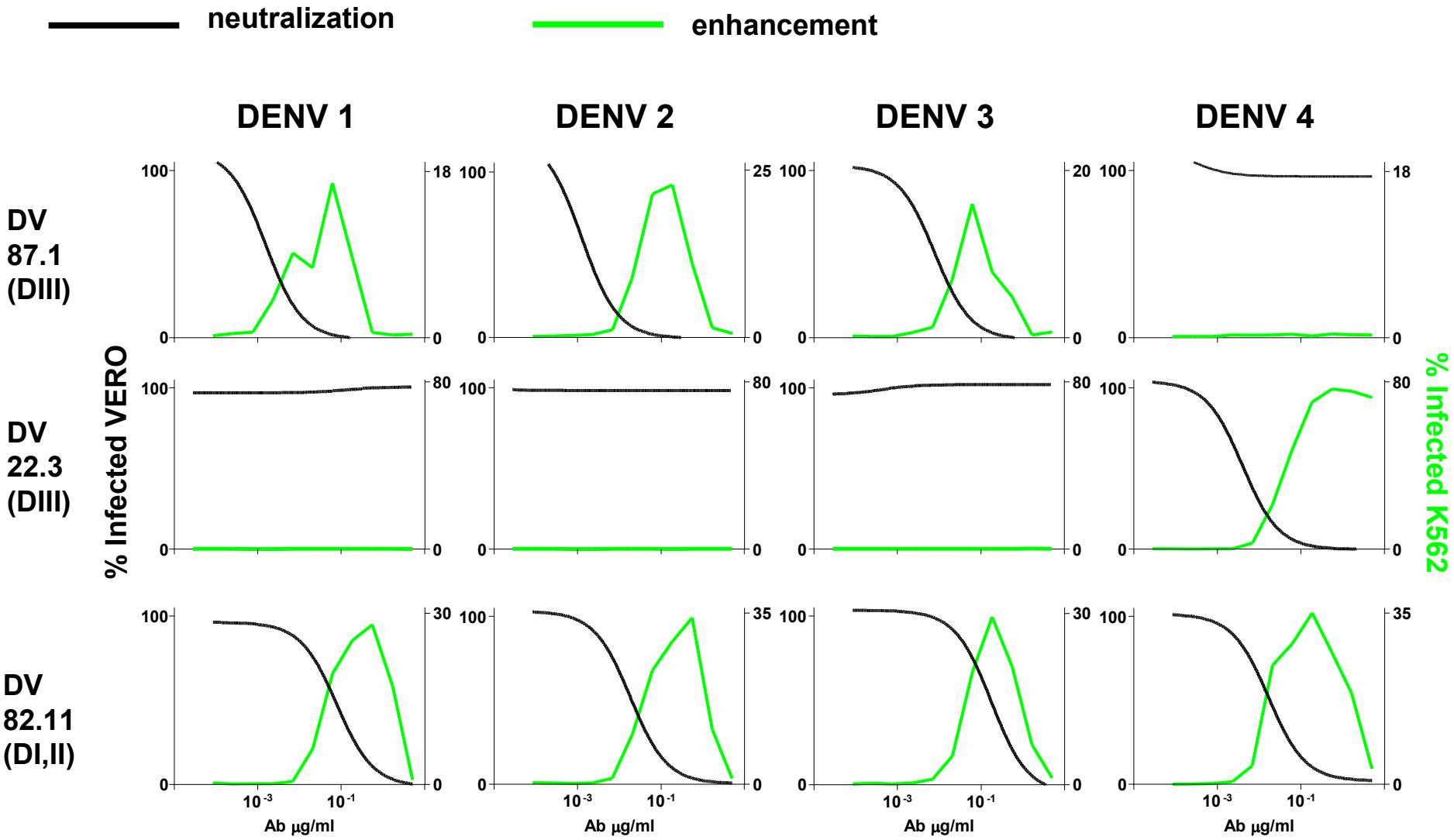
< 0.020 µg/ml
0.021-0.150 µg/ml
> 0.150 µg/ml

< 2 log
3 log
>4 log

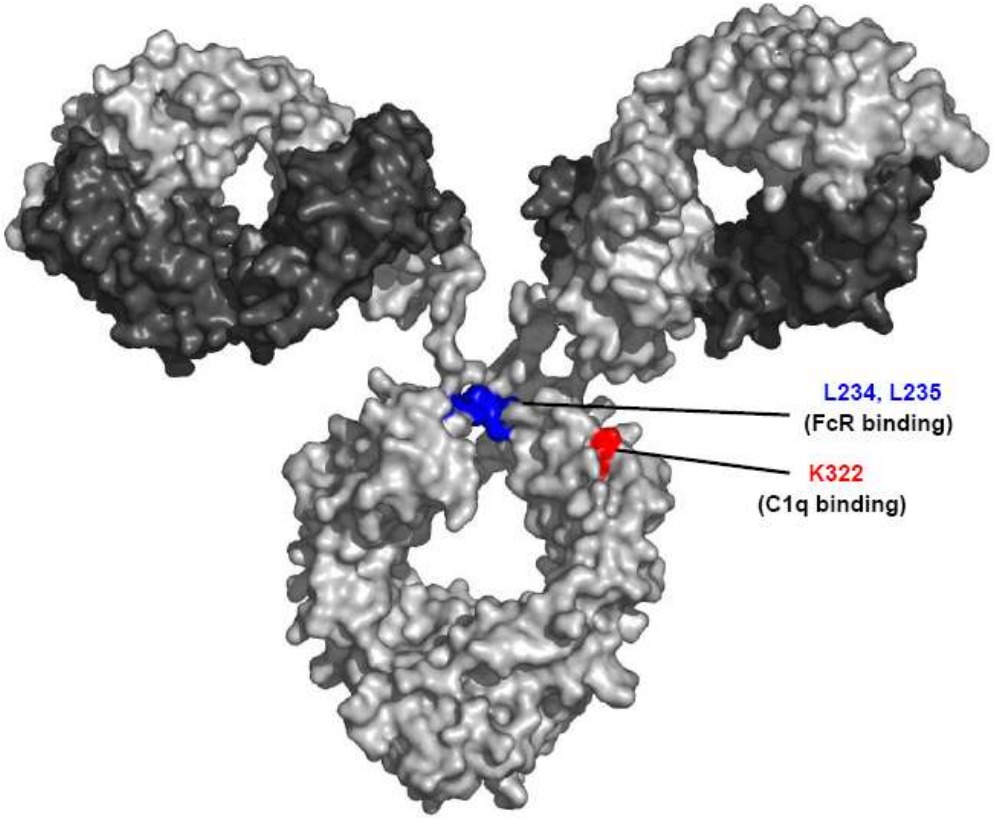
Human-mAbs for Passive Vaccination

- Should neutralize all four DENV serotypes
- Should target two or more non-overlapping regions on each virus (to avoid selection of escape mutants)
- Should not enhance infection and should inhibit the effect of enhancing antibodies

Three mAbs neutralizes the four DENV serotypes by binding two distinct epitopes on each virus

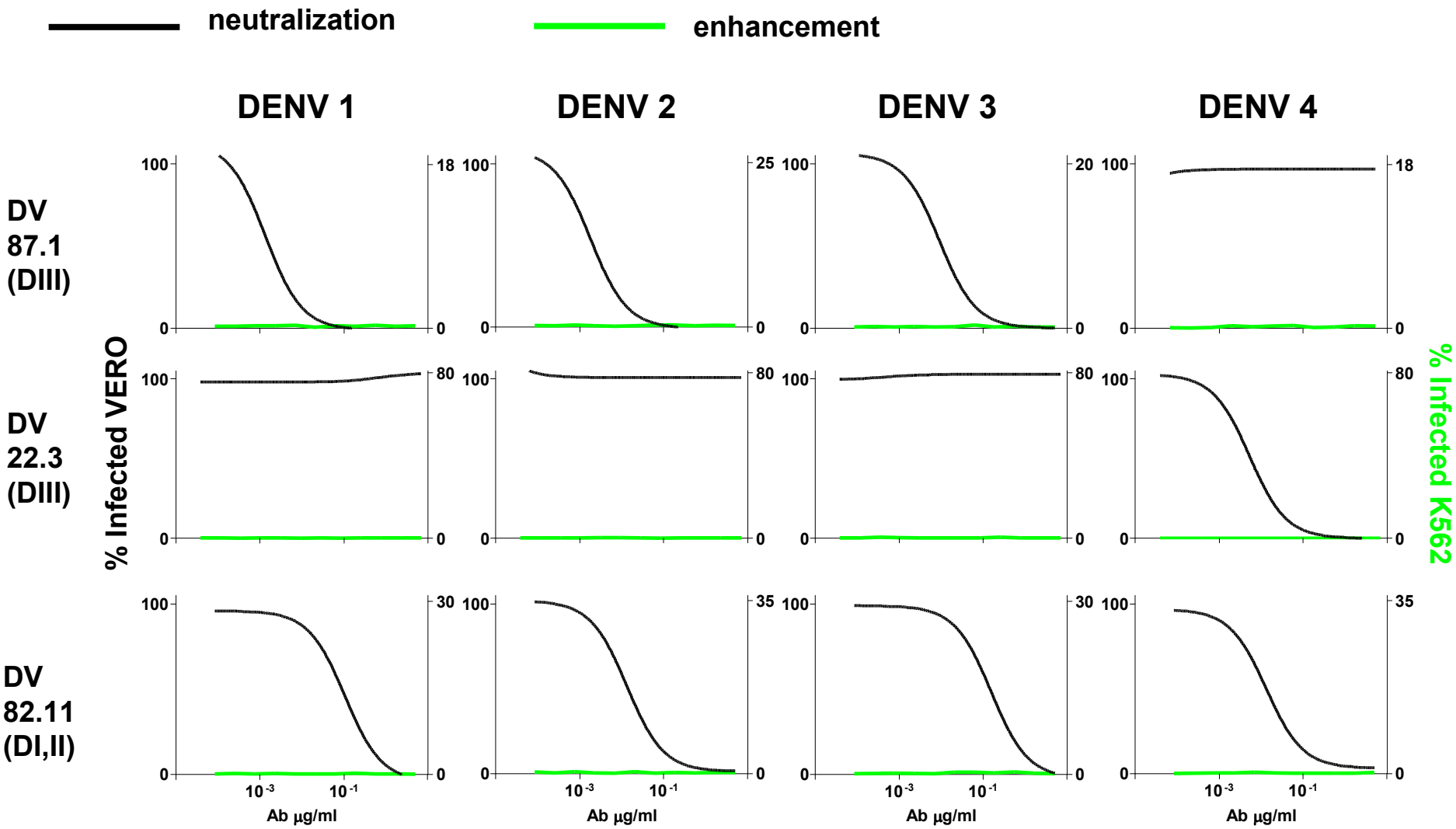


Two L to A substitutions in the CH2 domain (LALA) abolish Fc-R binding



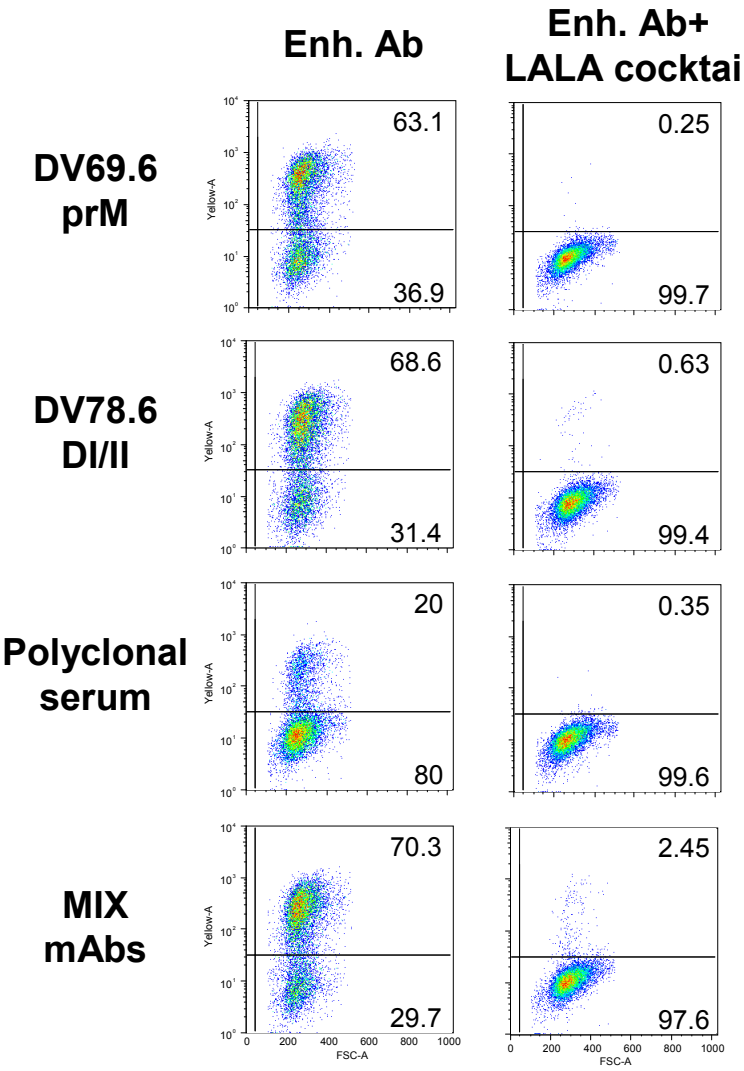
Parren et al. J. Vir 2001
Hessel et al. *Nature* 2007

LALA mutant mAbs retain full neutralizing capacity but do not enhance infection



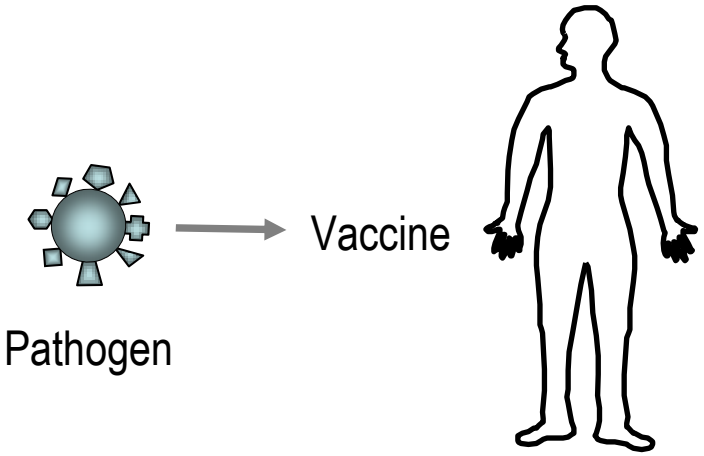
A mAb cocktail inhibits antibody dependent enhancement of infection

K562 -DENV3



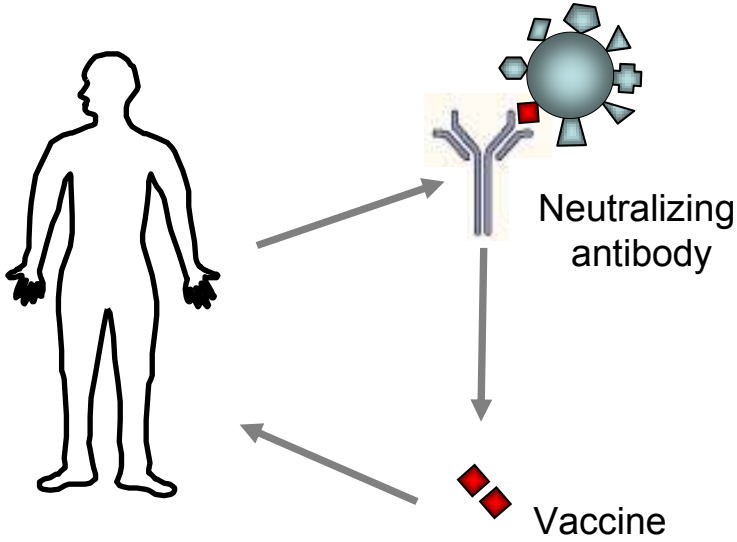
LALA cocktail:
DV82.11(DI/II)= 2 μg/ml
DV87.1 (DIII) = 0.2 μg/ml
DV22.3 (DIII) = 0.2 μg/ml

Classical Vaccinology



Killed / attenuated pathogen or a subunit are tested for their capacity to induce a protective antibody response.

Antibody-driven target discovery "Reverse/Analytic vaccinology"

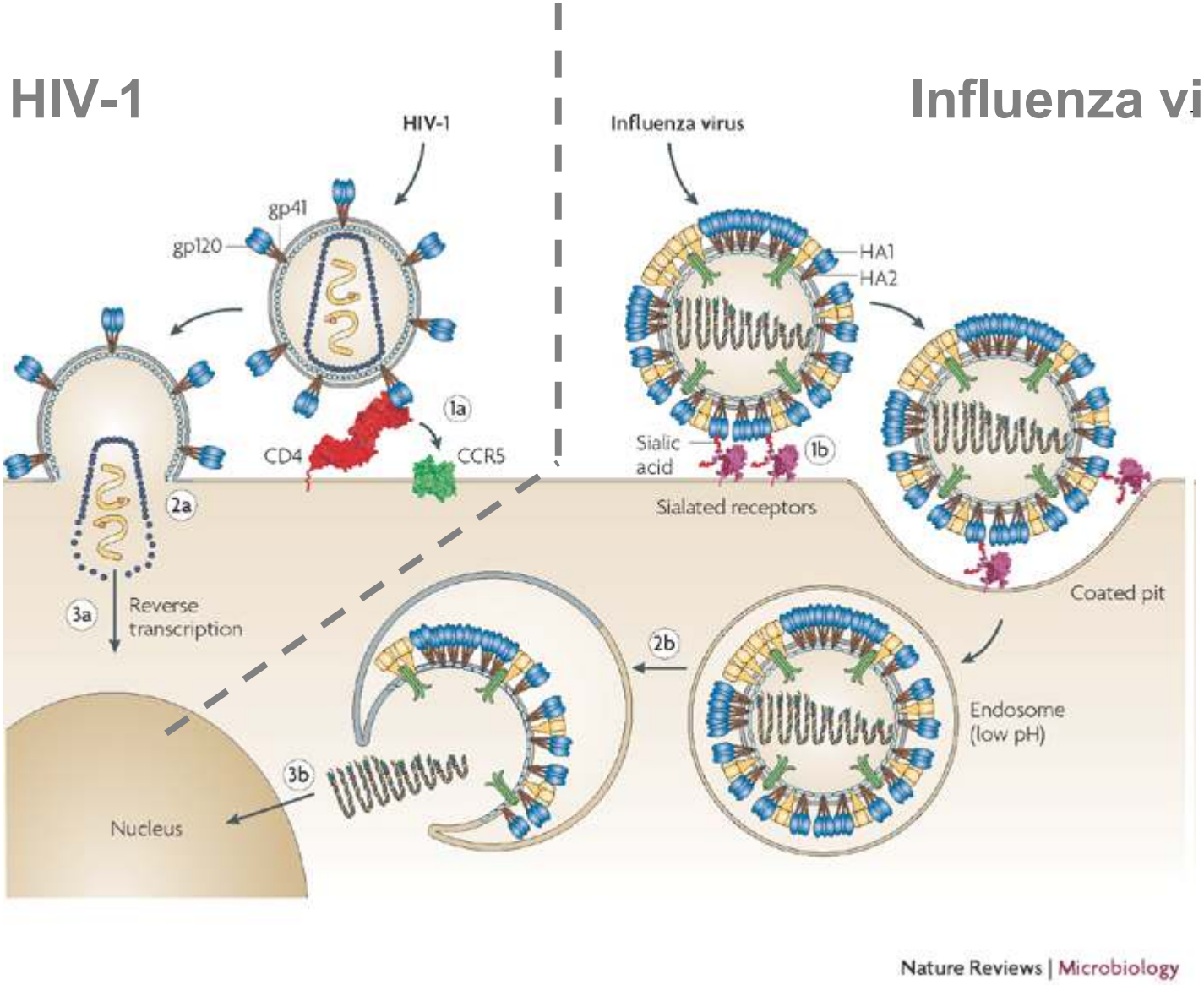


Burton, Nature Rev Immunol 2002

Neutralizing antibodies are used to discover target antigens that can be appropriately formulated as vaccine.

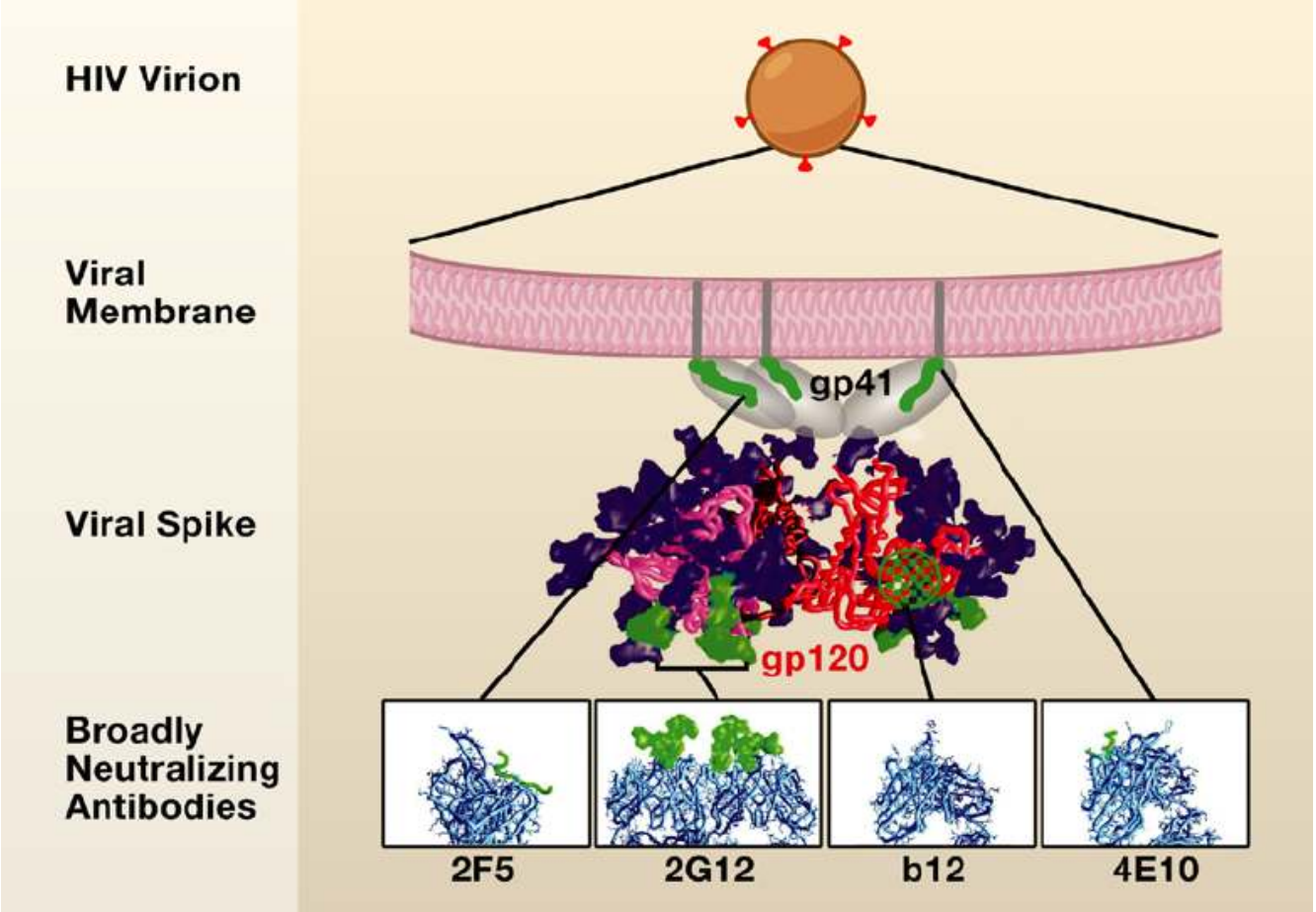
HIV-1

Influenza virus



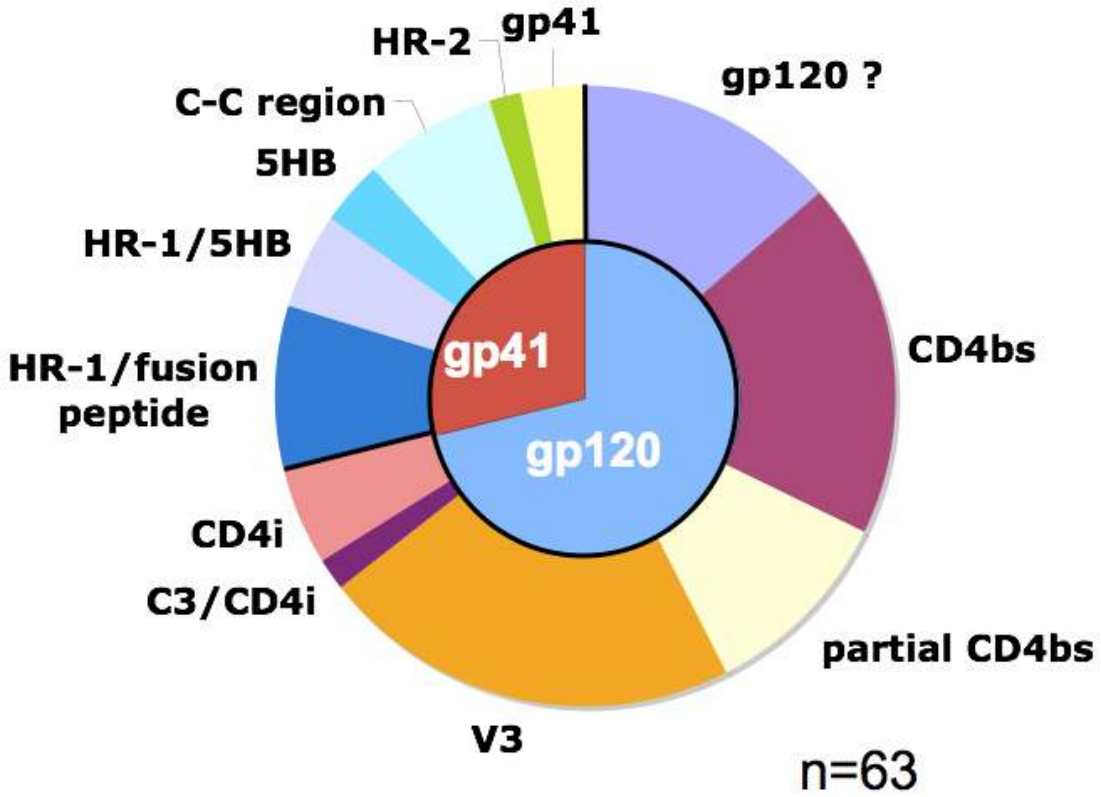
Nature Reviews | Microbiology

Neutralizing antibodies and HIV vaccine design



The Neutralizing Antibody Consortium

A panel of 63 monoclonal antibodies from memory B cells of HIV-infected individuals (CAVD, Weiss-VDC)

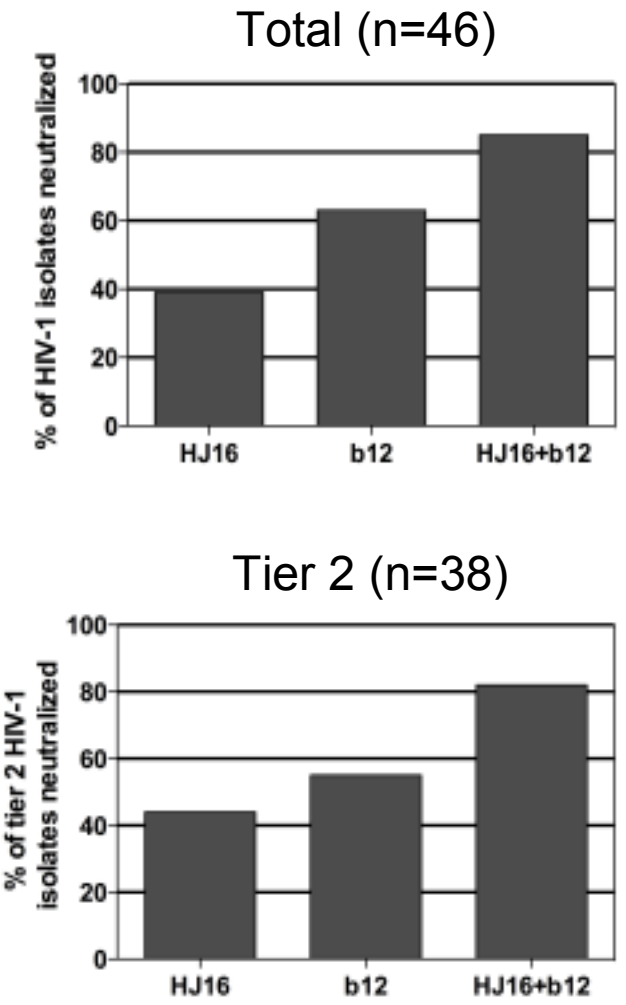


- 48/63 mAbs neutralize at least one isolate
- 14/48 mAbs neutralize isolates from more than one clade
- 3 mAbs are broadly neutralizing (CD4bs, HR1; V3 crown)
- None of the mAbs is MPER-specific or 2G12-like

HJ16: a CD4bs-specific mAb

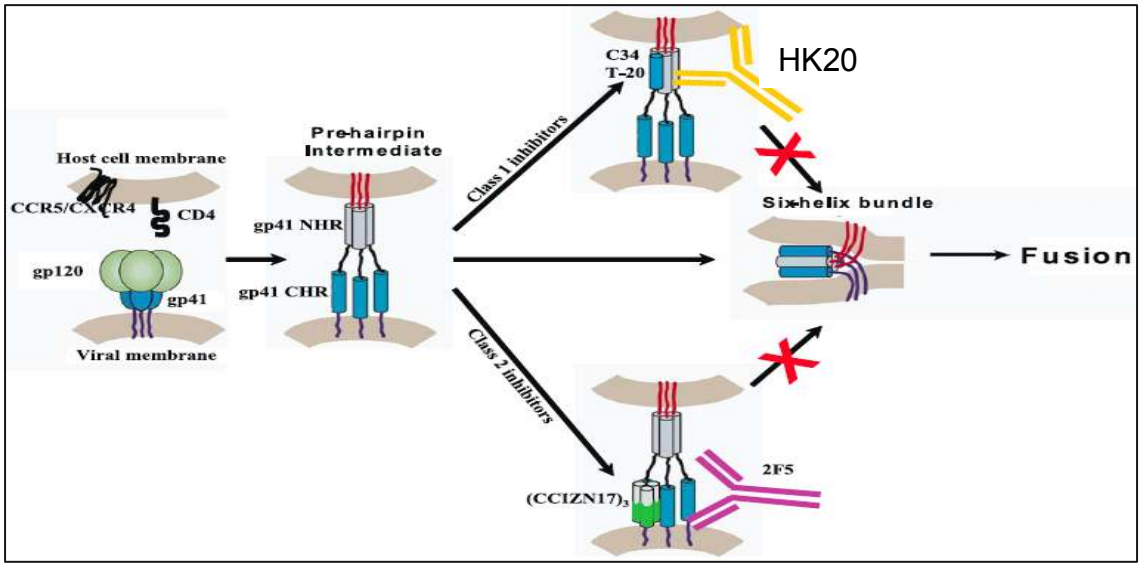
	HIV-1 isolates	Clade	Tier	b12	HJ16.22	
Tier 1	MS208.A1	A	1	1	>50	
	92RW020.2	A	1	19	1.90	
	SF162	B	1	0.01	>50	
	MN-3	B	1	0.1	>50	
	BaL.26	B	1	0.19	>50	
	SS1196.1	B	1	0.3	>50	
	BX08.16	B	1	4.2	>50	
	93MW965.26	C	1	0.2	>50	
Tier 2	Q769.d22	A	2	>50	>50	
	Q461.e2	A	2	>50	0.20	←
	Q259.d2.17	A	2	>50	>50	
	RHPA4259.7	B	2	0.1	0.01	
	SC42261.8	B	2	0.2	31.10	
	QH0692.42	B	2	0.3	16.40	
	THRO4153.18	B	2	0.5	>50	
	REJO4541.67	B	2	0.7	>50	
	6535.3	B	2	1.4	>50	
	AC10.0.29	B	2	1.9	>50	
	WITO4160.33	B	2	3.1	>50	
	CAAN5342.A2	B	2	>50	11.20	←
	TRO.11	B	2	>50	0.05	←
	PVO.4	B	2	>50	14.50	←
	TRJO4551.58	B	2	>50	>50	
	Du422.1	C	2	0.2	>50	
	CAP45.2.00.E8	C	2	0.7	>50	
	Du156.12	C	2	0.8	>50	
	Du172.17	C	2	1	23.70	
	Du151.2	C	2	1.4	>50	
	ZM214M.PL15	C	2	3	0.05	
	ZM249M.PL1	C	2	3.2	>50	
	ZM197MPB7	C	2	19.9	>50	
	CAP210.2.00.E8	C	2	20.4	>50	
	ZM53M.PB12	C	2	25.9	>50	
	ZM233M.PB6	C	2	>50	>50	
	ZM109F.PB4	C	2	>50	2.90	←
	96ZM651.2	C	2	>50	0.20	←
	ZM135M.PL10a	C	2	>50	>50	
	Q168.a2	CRF_A/D	2	>50	0.04	←
	T257-31	CRF02_AG	2	>50	17.00	←
	211-9	CRF02_AG	2	>50	3.90	←
	263-8	CRF02_AG	2	>50	>50	
	CH181.12	CRF07_BC	2	2.5	43.90	
	CH110.2	CRF07_BC	2	10.8	>50	
	CH064.20	CRF07_BC	2	>25	>50	
	CH111.18	CRF07_BC	2	>50	4.90	←
	271-11	CRF02_AG	2 (?)	38.7	0.10	

IC50 values (µg/ml). TZMbl-based assay (VIMC)

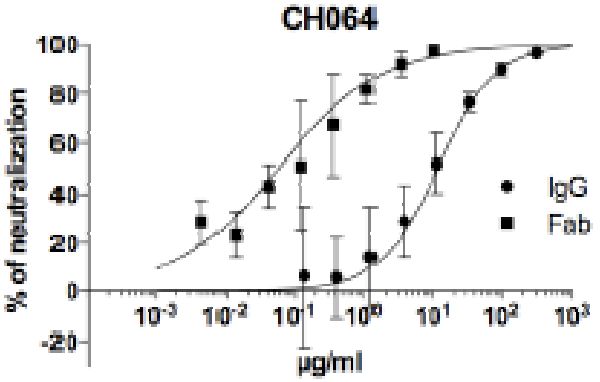


HK20: an HR1-specific mAb

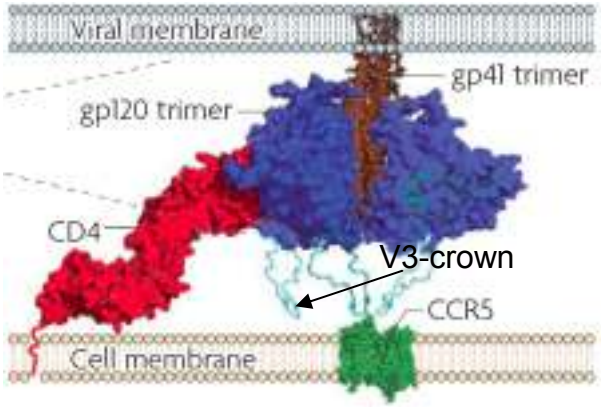
HIV-1 isolate	Clade	IC50 (µg/ml) IgG	Fab
Q461.e2	A	7.34	0.49
Q769.d22	A	9.22	0.11
Q168.a2	AD	40.85	0.12
T257-31	AG	35.04	0.16
263-8	AG	68.70	1.19
SF162	B	9.64	0.27
SC42261.8	B	10.03	0.15
CAAN5342.A2	B	9.63	0.09
JRFL	B	>300	>10
BaL	B	84.77	0.28
THRO4153.67	B	>300	0.48
H022.7	B	>300	5.70
CH181.12	BC	15.50	0.43
CH064.20	BC	11.10	0.06
ZM214M.PL15	C	5.84	0.01
ZM53M.PB12	C	15.44	0.60
ZM109F.PB4	C	14.14	0.15
CAP210.2.00.E8	C	11.89	0.16
Du151..2	C	16.62	0.61
ZM249.PL1	C	6.68	0.25
93MW965.26	C	8.50	0.08
96ZM651.2	B	1.46	0.04
VSV-G		>300	>10



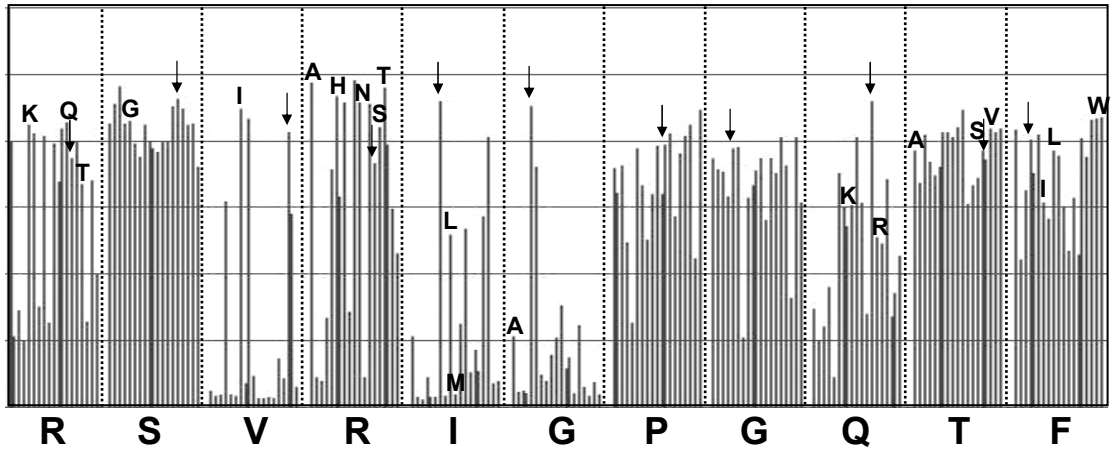
Modified from Hrin et al AIDS 2008



HGN194: a V3 crown-specific mAb



mAb	Epitope
HGA13	- - - - - - I G P G Q T F Y - - -
HGI95	- - K S I R I G P - - - - - - - -
HGD65	- - - - - - - G P G R A F Y - - -
HGN194	- R R S V R I G P G Q T F - - - -
HR10	C R R S V R I G P G Q T F Y A T C
HGA49	- - - - - - I G P G Q T F Y A - -
HGA9	- - - - V R I G P G Q T F Y - - -
HZ74	- - R S V R I G P G Q T F Y - - -



- HGN194 neutralizes all tier 1 isolates tested and several tier 2 isolates
- Aminoacid replacement analysis is consistent with its broad reactivity

Extensive crossclade neutralization by a cocktail of mAbs

		mAbs (% of neutralization)			
	specificity	V3	HR1	CD4bs	1:1:1
	HIV-1 isolates	HGN194	HK20	HJ16	Cocktail
A	Q461.e2	67	87	100	100
	Q769.d22	83	76	<50	97
	92RW020.2	75	78	92	100
	MS208.A1	94	70	<50	97
	Q259.d2.17	81	<50	<50	93
B	BaL.26	100	<50	<50	100
	SF162	100	68	<50	100
	RHPA4259.7	72	<50	100	100
	SS1196.1	100	71	<50	100
	AC10.0.29	64	75	<50	96
	H022.7	<50	<50	<50	58
	CAAN5342.A2	<50	65	79	98
	TRO.11	<50	<50	97	95
	TRJO4551.58	<50	<50	<50	57
C	SC4226.8	<50	62	<50	97
	ZM214.PL15	<50	78	99	100
	ZM109F.PB4	97	86	82	100
	CAP45.2.00.E8	<50	70	<50	78
	93MW965.26	100	88	<50	100
	96ZM651.2	77	94	91	100
	Du151.2	<50	74	90	100
	Du156.12	<50	81	<50	91
	ZM249M.PL1	74	81	<50	98
AD	Q168.a2	<50	58	100	100
AG	T257-31	66	78	96	98
	263-8	83	58	<50	95
BC	CH181.12	83	75	92	100
	CH110.2	65	84	<50	97
	VSV-G	<50	<50	<50	<50

HOS-based assay

Prospects for a universal influenza vaccine

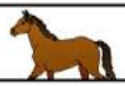
H1 and **H3** subtypes cause disease in humans and continuously drift. **H5**, **H7** and **H9** subtypes cause sporadic cases and may generate a new pandemia

The neutralizing antibody response to influenza virus is thought to be **subtype specific**

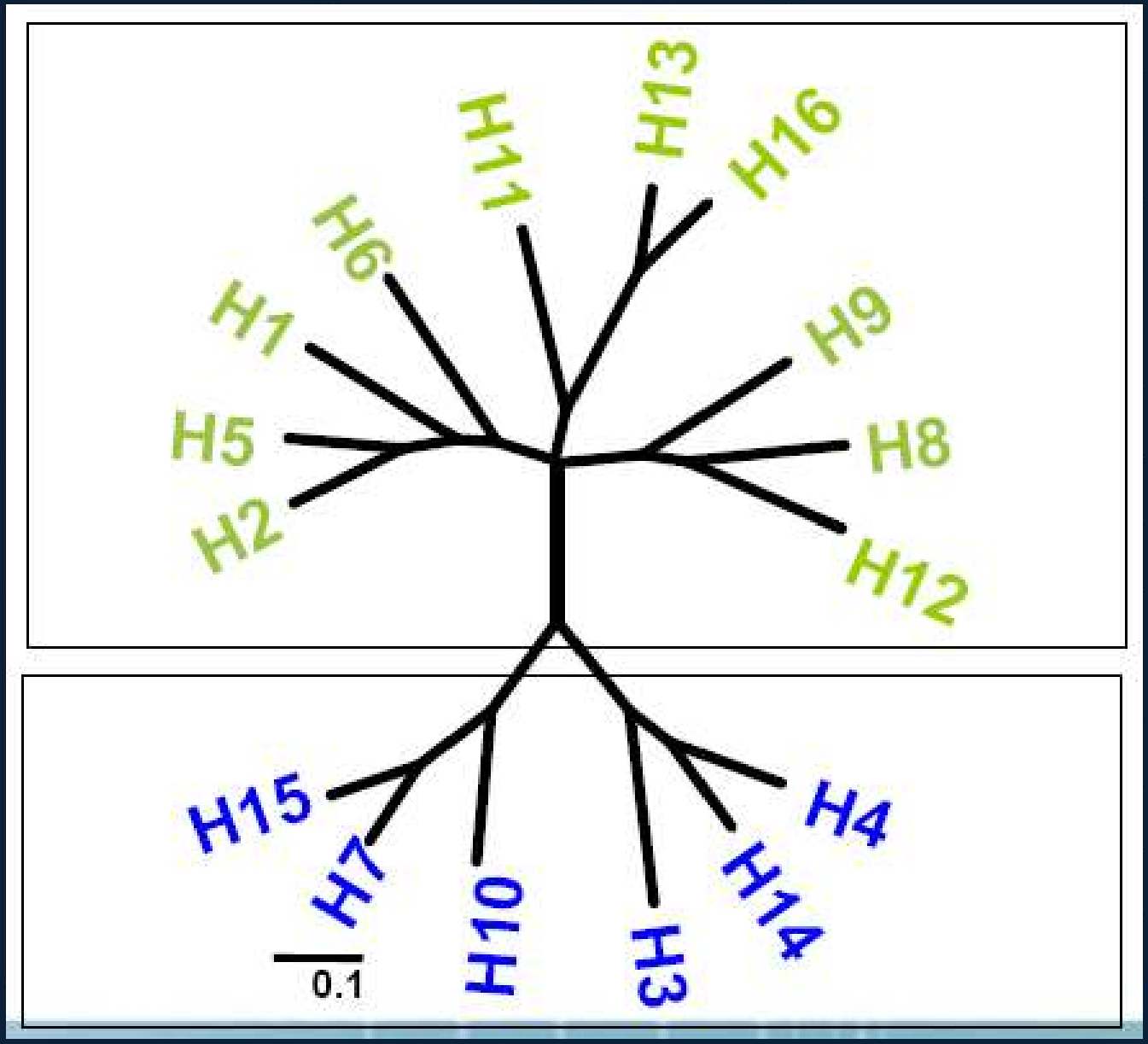
Recently **broadly neutralizing antibodies** have been isolated from phage libraries
(Thorsby et al PLoS One 2008; Sui et al Nature Str & Mol Biol 2009; Ekiert et al Science 2009)

Are these antibodies produced in the course of the immune response to Flu?

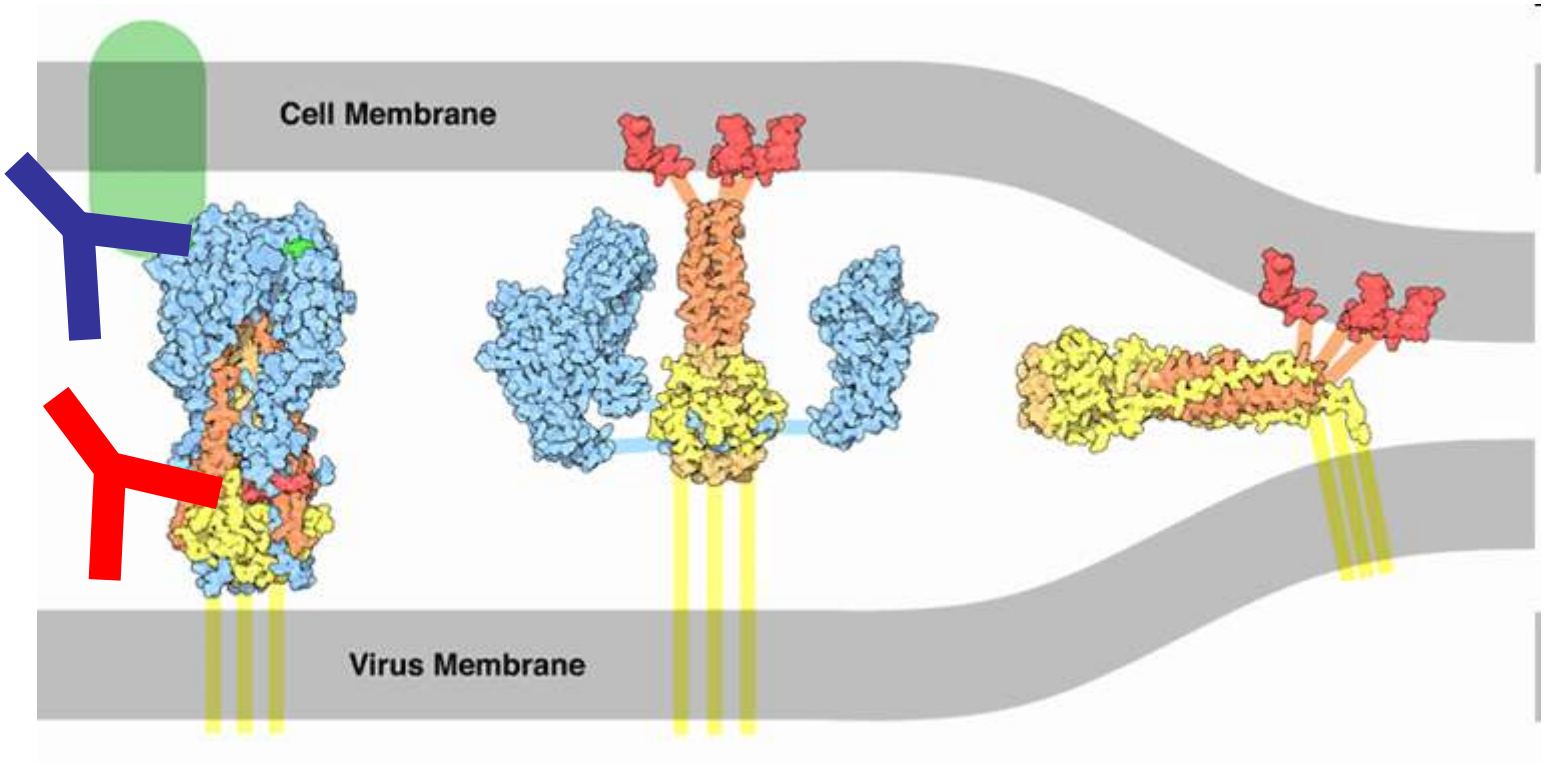
Where Do These Non-Human Viruses Come From?

H1	  
H2	 
H3	  
H4	 
H5	
H6	
H7	  
H8	
H9	
H10	
H11	
H12	
H13	
H14	
H15	

Where Do These Non-Human Viruses Come From?



Two types on influenza neutralizing antibodies

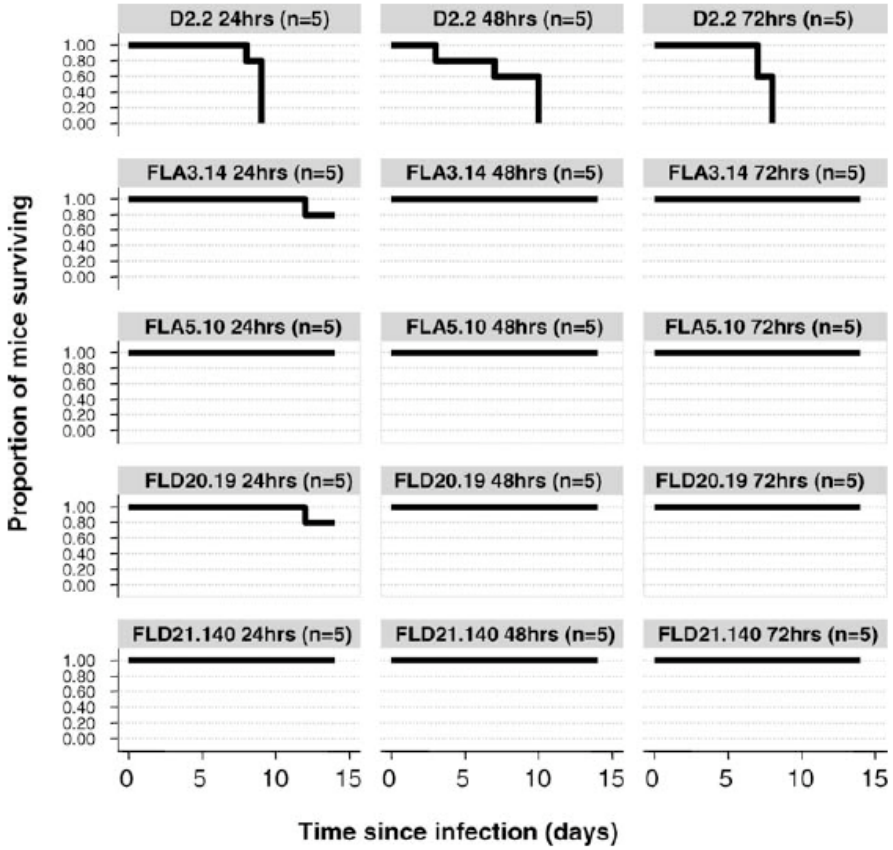


Bind to variable epitopes in the globular head and inhibit hemagglutination

Bind to conserved epitopes in the stem and block fusion Use VH1-69

Prophylactic and therapeutic efficacy of human monoclonal antibodies against H5N1 influenza

Human mAbs were administered i.p.
24, 48 or 72 hours post infection



Simmons et al. PLoS Medicine , 2007

Broad and potent neutralization of H5N1 isolates

H5-pseudotype neutralization: IC90 (µg/ml)

Clade	1	1	1	2.1	2.2	2.2	2.3
HA	A/HK/213/03	A/VN/1203/04	A/VN/1194/04	A/IN/5/05	A/WS/Mong/244/05	A/Ty/Ty/1/05	A/Anhui/1/05
FLD194	0.007 *	0.023	0.019	0.033	0.008	0.019	0.131
FLD122	0.009	0.039	0.031	0.033	0.045	0.033	0.070
FLD20	0.033	0.6	0.144	0.149	0.345	0.355	0.607
FLA3	0.090	0.660	0.221	1.230	0.169	0.188	0.514
FLD129	0.0015	0.008	0.007	nn	0.010	0.029	0.027
FLD84	0.117	0.240	0.357	0.280	0.293	0.117	0.195
FLD93	0.051	0.335	0.209	0.135	0.137	0.059	nn
FLD132	0.001	0.004	0.006	nn	0.004	0.008	nn
FLD21	0.006	0.015	0.033	nn	0.031	0.006	nn
FLA5	0.01	0.124	0.075	nn	nn	nn	nn
FLD127	nn	0.183	0.167	nn	nn	nn	nn

*** When administered at 1mg/Kg could confer a 90% serum neutralizing titer >1/200**

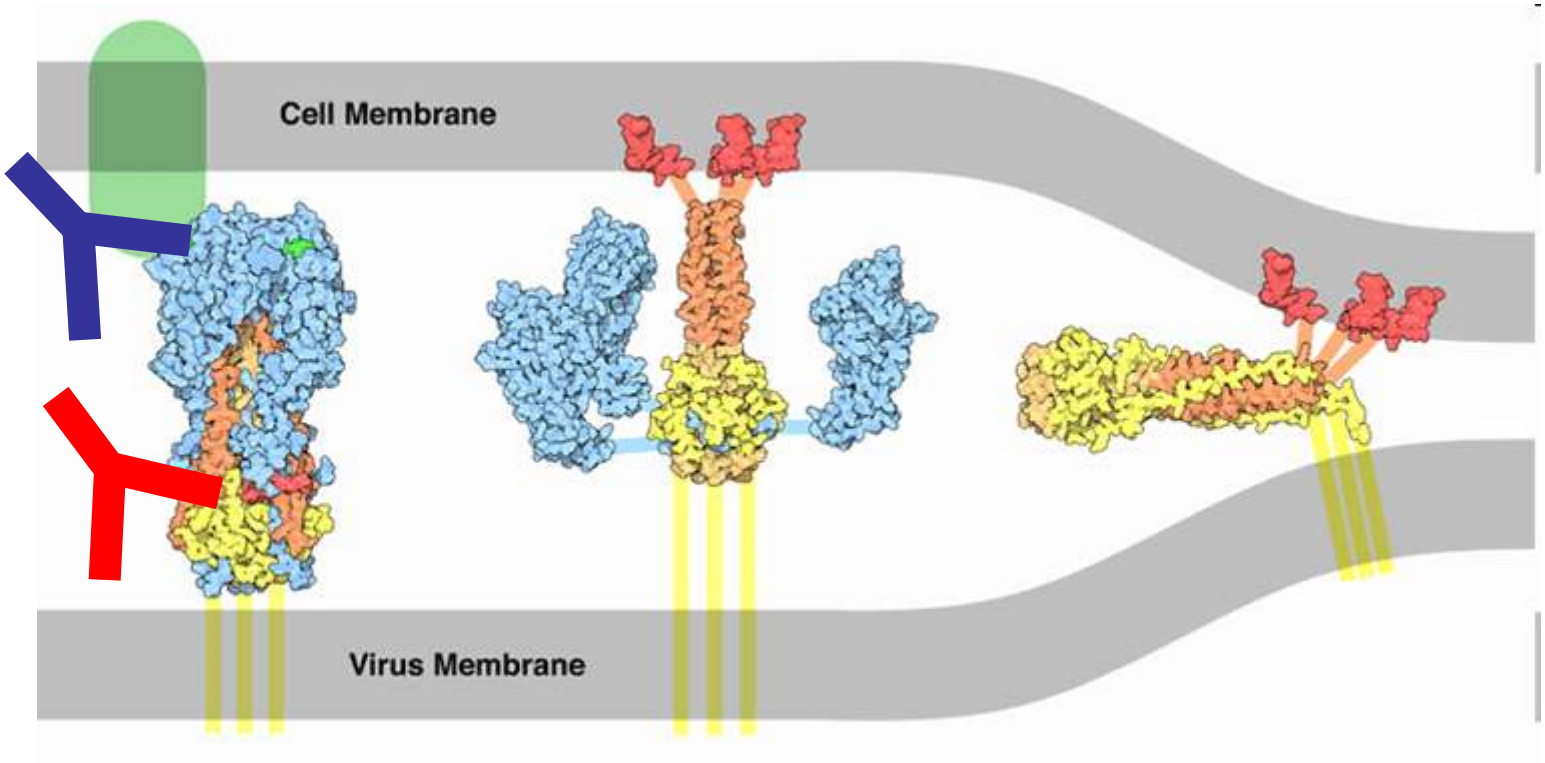
Collaboration with Nigel Temperton and Robin Weiss

A “cocktail” of mAbs recognizing non-overlapping epitopes to prevent selection of escape mutants

Competing mAb	FLD194-bio	FLA3-bio	FLD20-bio
FLD194	100*	0	0
FLA3	100	100	0
FLD20	0	0	100
FLA5	100	0	100
FLD21	0	0	100
FLD132	52	0	0
FLD93	0	0	100
FLD84	0	0	100
FLD127	0	0	100
FLD122	100	65	100
FLD129	100	0	100

* % inhibition of biotinylated antibody by an excess of cold antibody

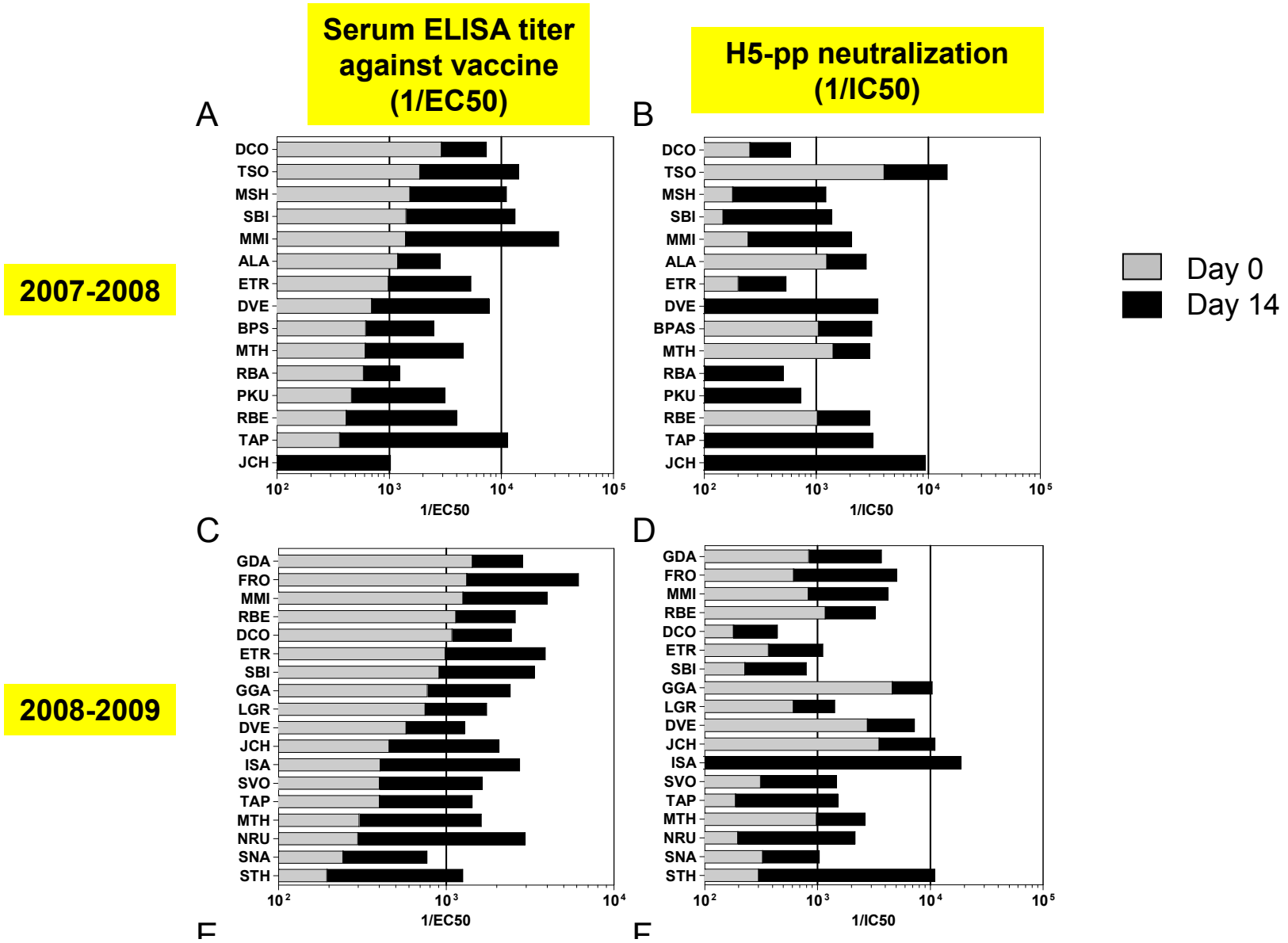
Two types on influenza neutralizing antibodies



Bind to variable epitopes in the globular head and inhibit hemagglutination

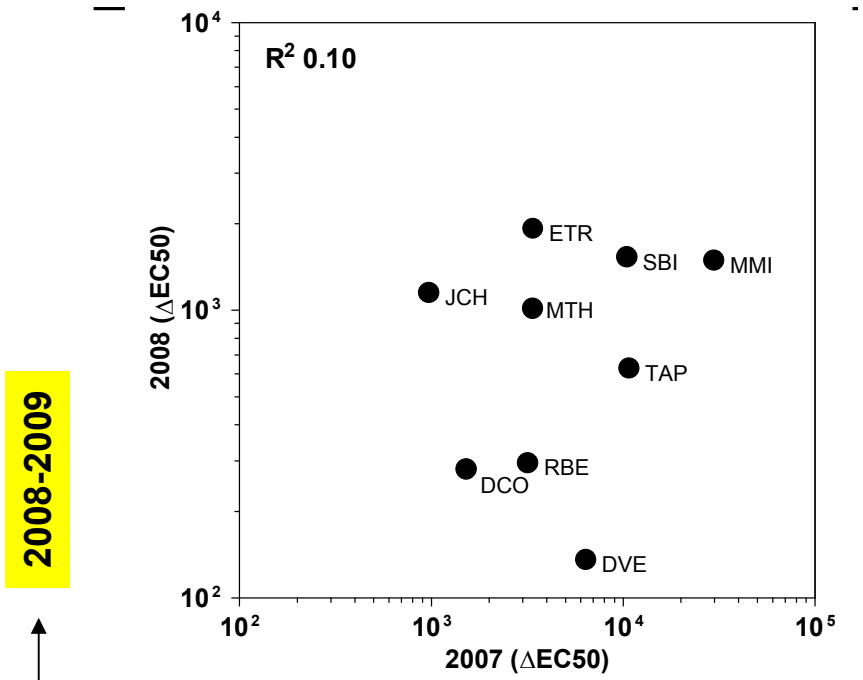
Bind to conserved epitopes in the stem and block fusion Use VH1-69

An unexpected responses to seasonal influenza vaccine

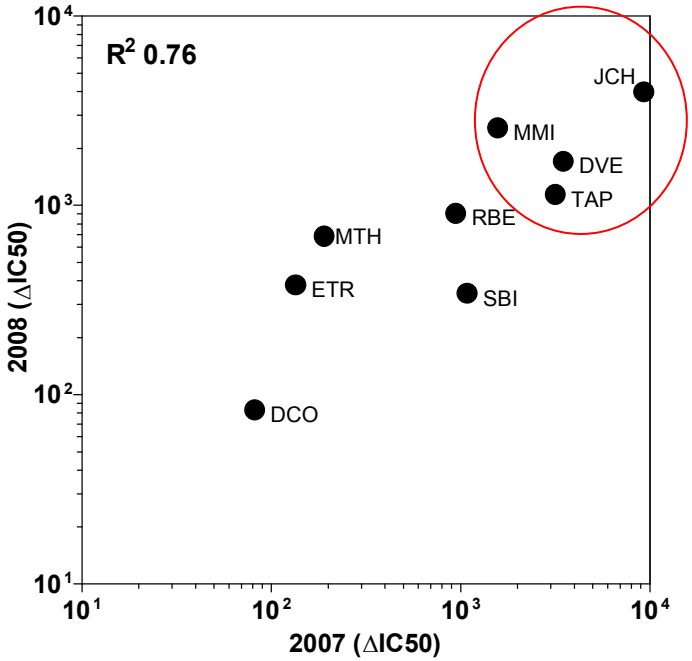


An unexpected responses to seasonal influenza vaccine

Serum ELISA titer against vaccine (1/EC50)



H5-pp neutralization (1/IC50)



2008-2009

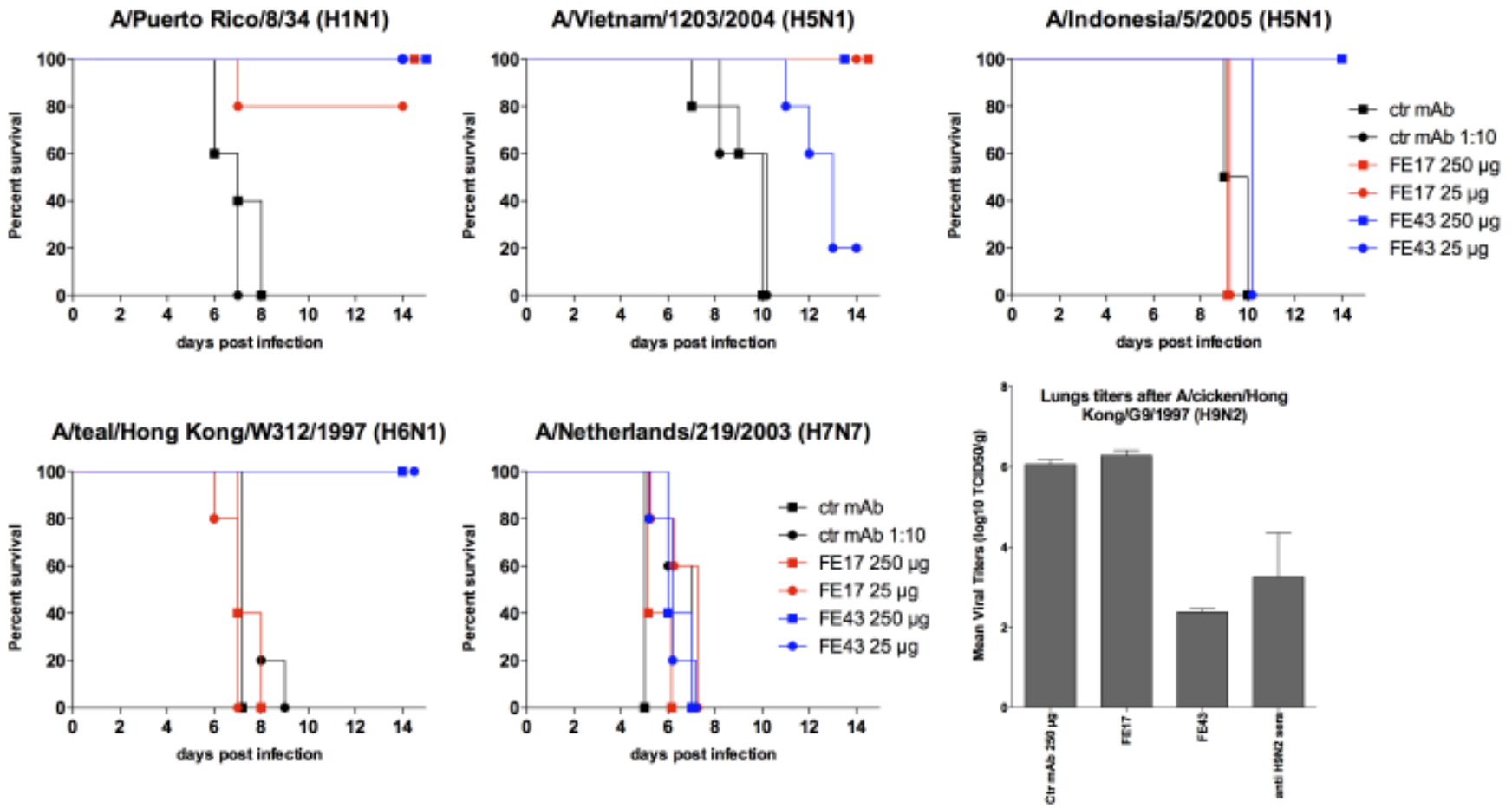
2007-2008

Human mAbs neutralizing different influenza subtypes

	ELISA				H5-HA staining			IC90 (µg/ml)		IC50 (µg/ml)			
	H1	H5	H9	H7	Ctrl	Acid treated	C179 competition	H5pp VN1194	H5pp IN05	H1N1 SI-06	H5N1 VN04	H9N2 HK97	H7N7 NE99
FE17	+	+	-	-	+	+	-	0.30	>50	<0.04	6.20	>50	>50
FB110	+	+	-	-	+	-	+	0.01	1.64	22.00	36.20	>50	>50
FB15b	+	+	-	-	+	-	+	0.19	>50	7.80	>50	>50	>50
FB54	+	+	+	-	+	-	+	0.04	0.43	50.00	>50	8.80	>50
FB75	+	+	-	-	+	-	+	0.00	0.36	40.00	>50	>50	>50
FC1c	+	+	-	-	+	-	+	0.02	19.20	>50	>50	50.00	>50
FC41	+	+	+	-	+	-	+	0.01	0.02	31.20	40.00	8.80	>50
FC6	+	+	+	-	+	-	+	0.04	2.00	17.50	>50	8.80	>50
FE43	+	+	+	-	+	-	+	0.03	0.02	8.80	>50	4.40	>50
FG20	+	+	-	-	+	-	+	1.56	nd	>50	>50	>50	>50
FC98	+	-	-	-	+	+	-	>50	>50	<0.04	>50	>50	>50

*ELISA with recombinant glycoproteins
^H5-pp = H5 pseudoparticles

Passive immunization and survival of mice after Influenza virus challenge



Data from Kanta Subbarao, NIAID

Heterosubtypic neutralizing antibodies

In response to seasonal influenza vaccination some individuals produce antibodies that neutralize multiple influenza subtypes

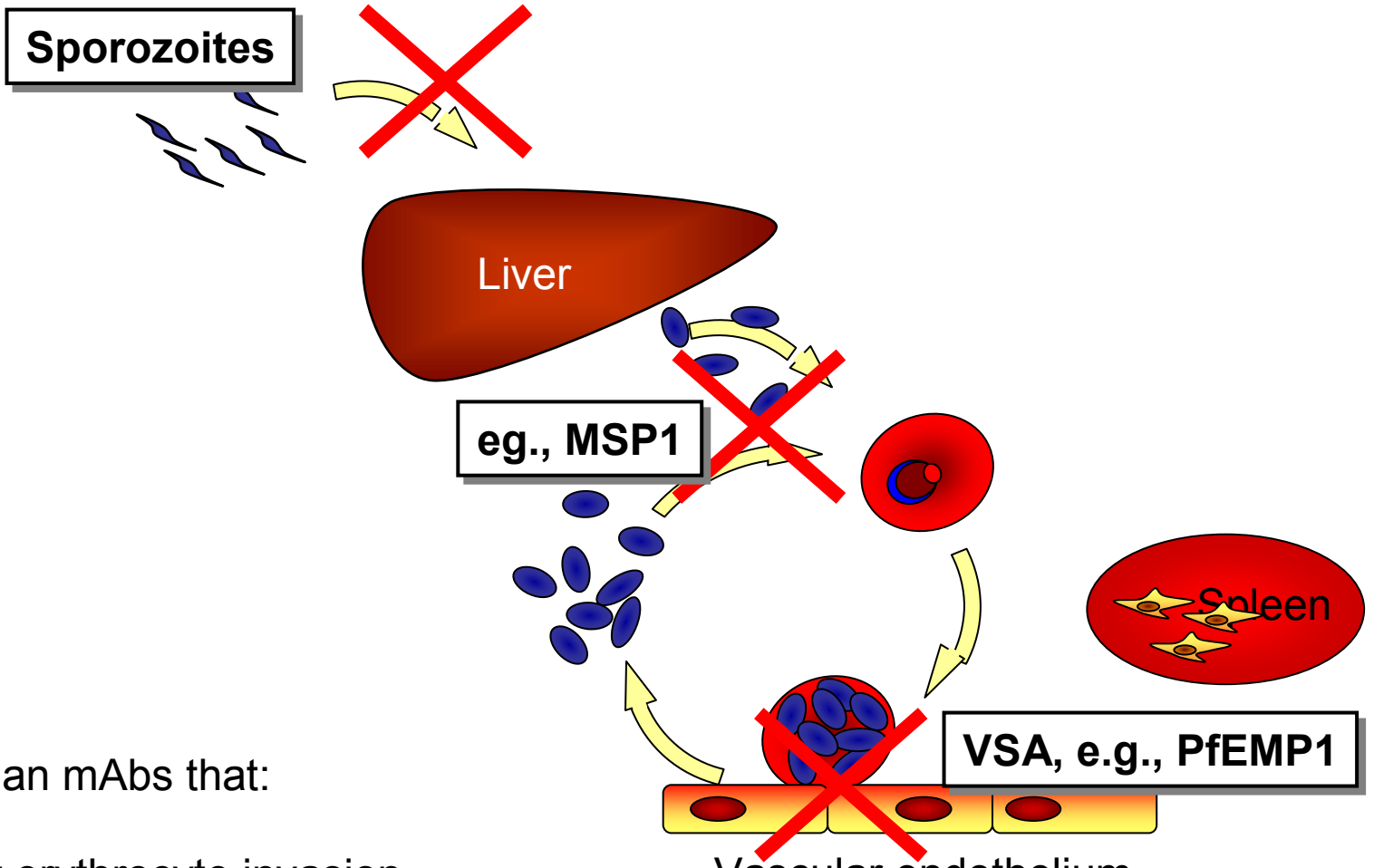
The frequency of heterosubtypic memory B cells ranges from 0.1 to 50% of vaccine specific B cells

>200 mAbs show broad neutralizing activity in vitro (and in vivo) against several group 1 viruses (H1, H2, H5, H6, H9). One mAb neutralizes both group 1 and group 2 inflluenza viruses

Most of these antibodies utilize VH1-69, recognize an acid labile epitope in the stem region and inhibit fusion rather than hemagglutination

Attempts to isolate escape mutants were unsuccessful

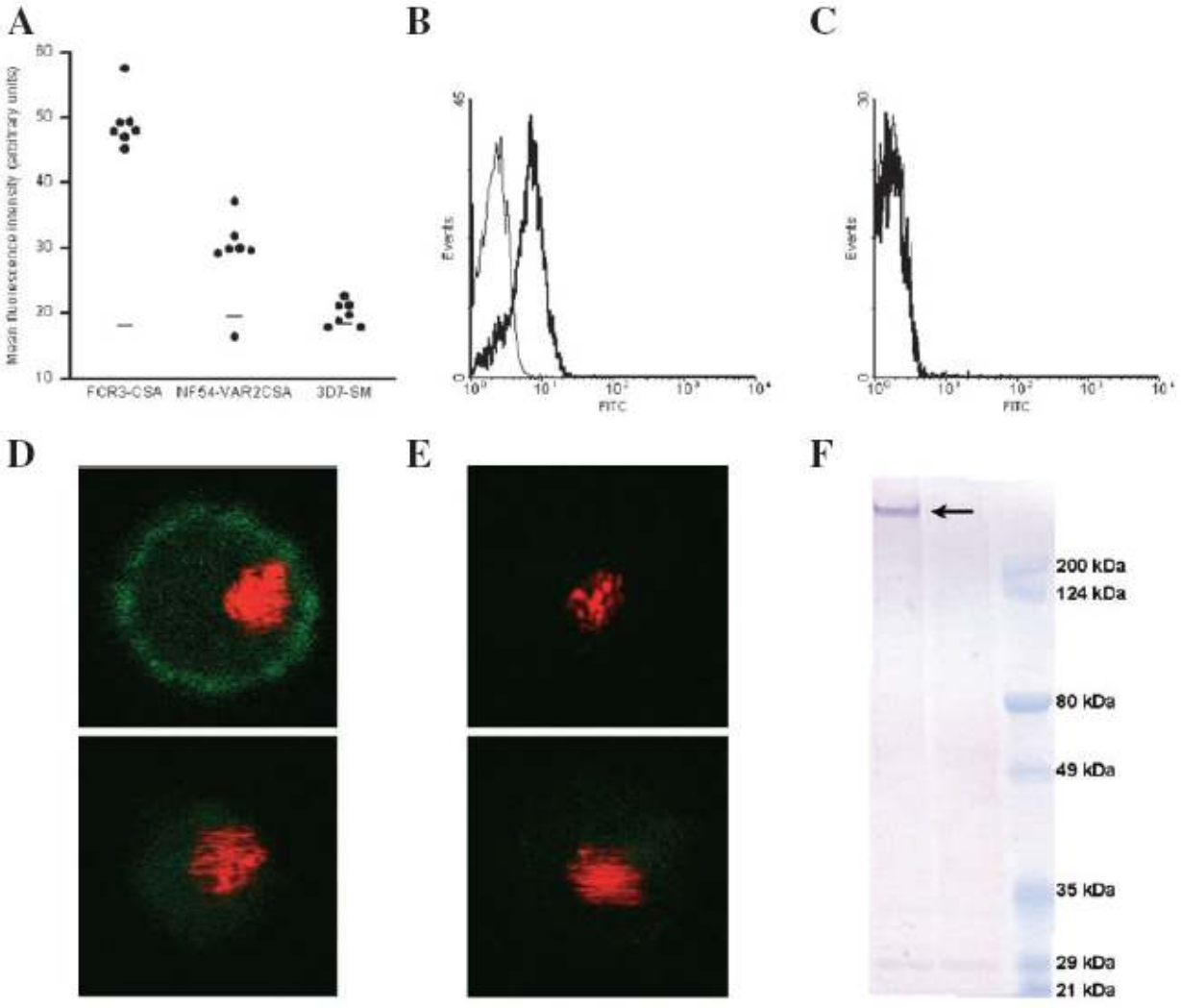
Potential targets of protective antibodies in Malaria



Human mAbs that:

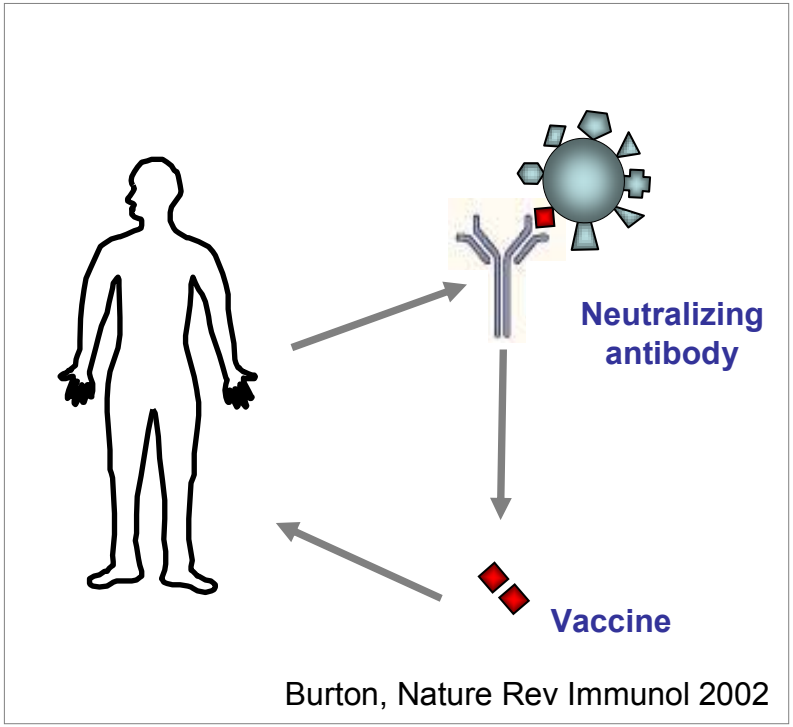
1. Inhibit erythrocyte invasion
2. Stain infected RBC
3. Inhibit adhesion of infected RBC

Human IgG monoclonal antibodies to VAR2CSA



Barfod L et al *Mol Microbiol*, 2007

Human monoclonal antibodies and global health



Burton, Nature Rev Immunol 2002

Dengue Virus: neutralizing and infection enhancing antibodies

Influenza: heterosubtypic neutralizing antibodies

HIV-1: epitopes recognized by broadly neutralizing antibodies

Cytomegalovirus: ligands responsible for cellular tropism

P. falciparum: antibodies that recognize infected RBC or inhibit invasion

Bacteria and fungi: epitopes targeted by "neutralizing" antibodies

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Davide Corti (HIV, Flu)
Annalisa Macagno (CMV)
David Jarrossay (Plasma cells)
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