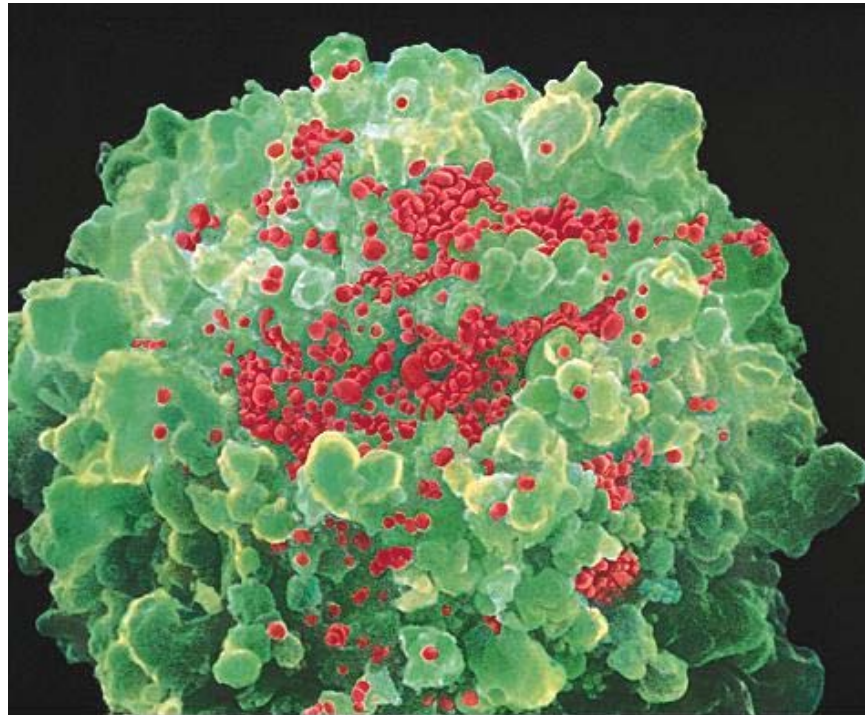
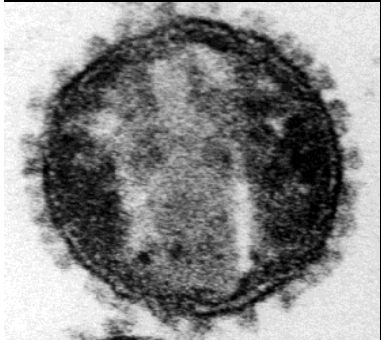


The Nature of Protective Antibodies

Robin A. Weiss





HIV positive individuals have weak but broad neutralising activity

Virus	n	Mean titer of antisera		
		ELISA	IFA	Neutralisation
HTLV-1	9	1216	2250	3257
HIV-1	12	1272	2847	~50

(Weiss et al, Nature 316: 69-72, 1985)

Antisera raised against recombinant gp120 neutralised only the same strain of HIV

(Weiss et al, Nature 324: 572-575, 1986)

Protective and Non-Protective Antibodies

- **Lessons from other infections**
- **Neutralizing antibodies**
- **Non-neutralizing antibodies**
- **Effector mechanisms**
- **Harmful antibodies?**

Neutralization as a Correlate of Protection

Rolf Zinkernagel (*Science* 2003)

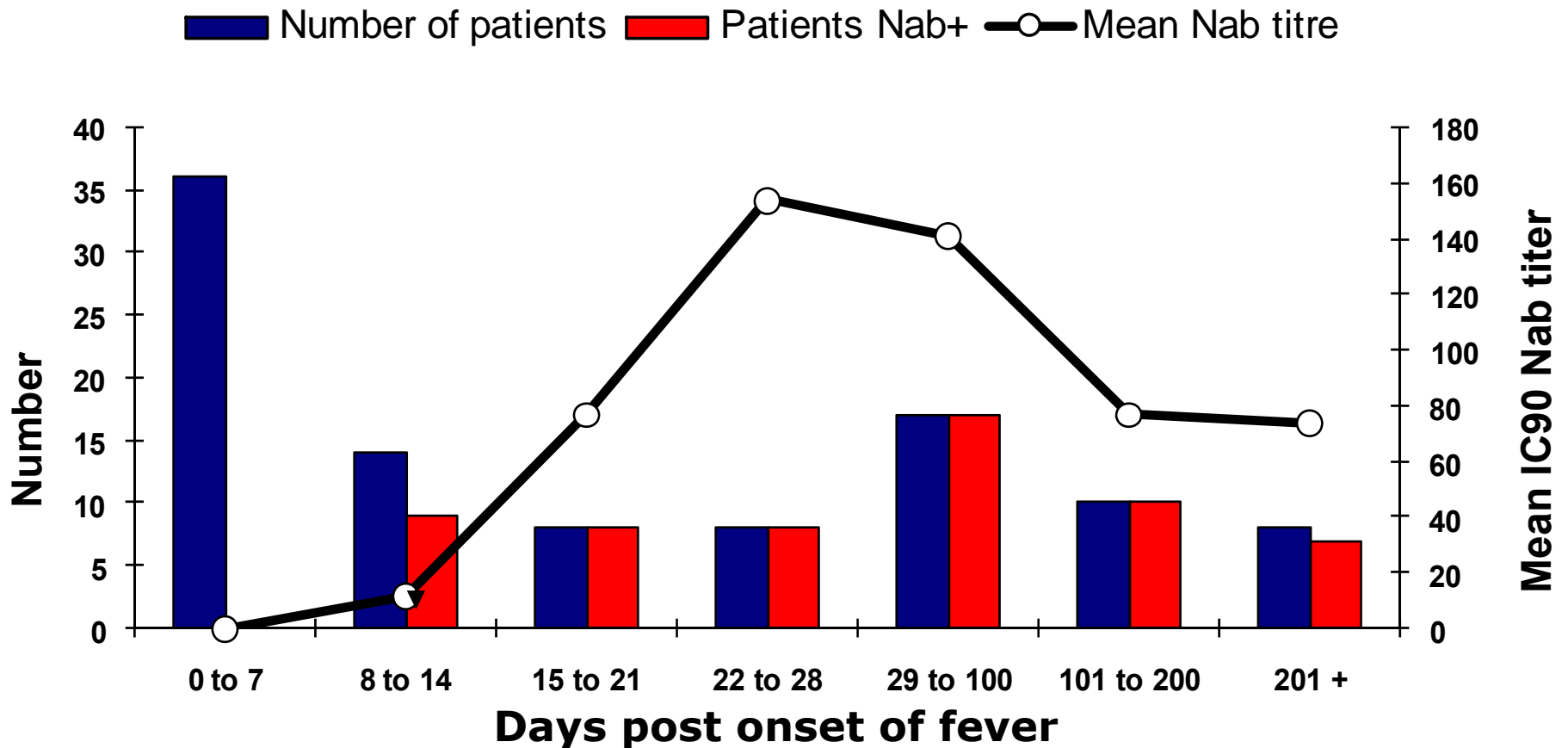
- **All virus vaccines to date elicit neutralizing antibodies**
- **HIV elicits poor neutralizing antibodies**
- **Will efficacious HIV vaccines ever be realized?**
- **Isn't the Global HIV Vaccine Enterprise pie in the sky?**

Virus vaccines to date elicit protective antibodies

- **Smallpox, Yellow fever, Rabies, MMR**
- **Polio: serotypes 1, 2 & 3**
- **Influenza: neutralization and hemagglutination inhibition (HI)**
- **Hepatitis B**
- **HPV**

- **WHO reference criteria for protection are based on antibody responses**
- **But cell-mediated immunity is seldom studied**

Neutralizing Titers to SARS Coronavirus (using an HIV pseudovirus assay)

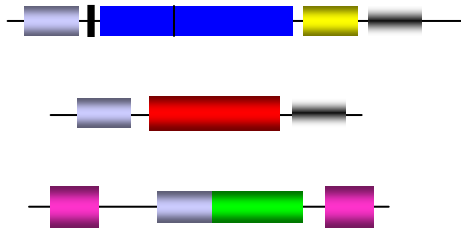


Temperton *et al*, *Emerg Infect Dis* 11: 411-416, 2005

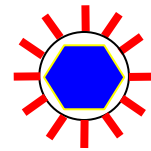
Studies with Xiao-Ning Xu on 128 recovered SARS patients show only 40% with CTL responses but 98% with neutralizing antibodies (Li *et al*, 2007)

Pseudotype Neutralisation Assay for H5N1 HA

1. Three plasmid transfection of 293T cells

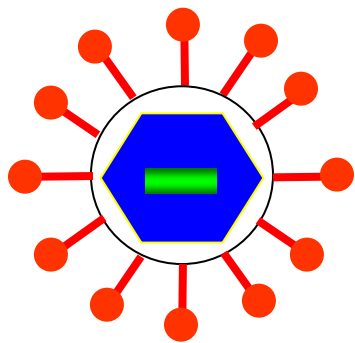


2. Add neurominidase and harvest pseudotype virus: titre 8×10^6



3. Incubate pseudotype with Ab and infect cells

4. Count fluorescent cells or rlu

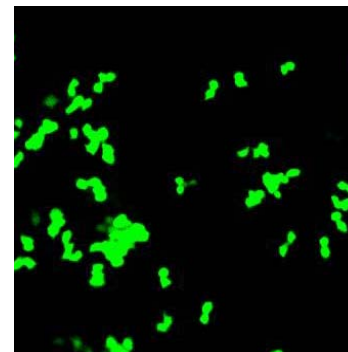


H5N1 (HA)

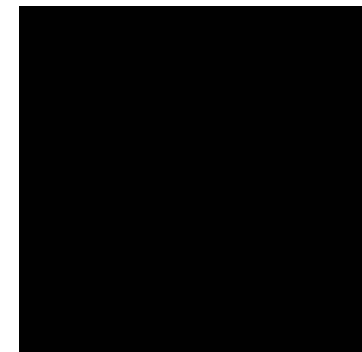
HIV gag and pol

Vector eGFP or Luc

No Antiserum

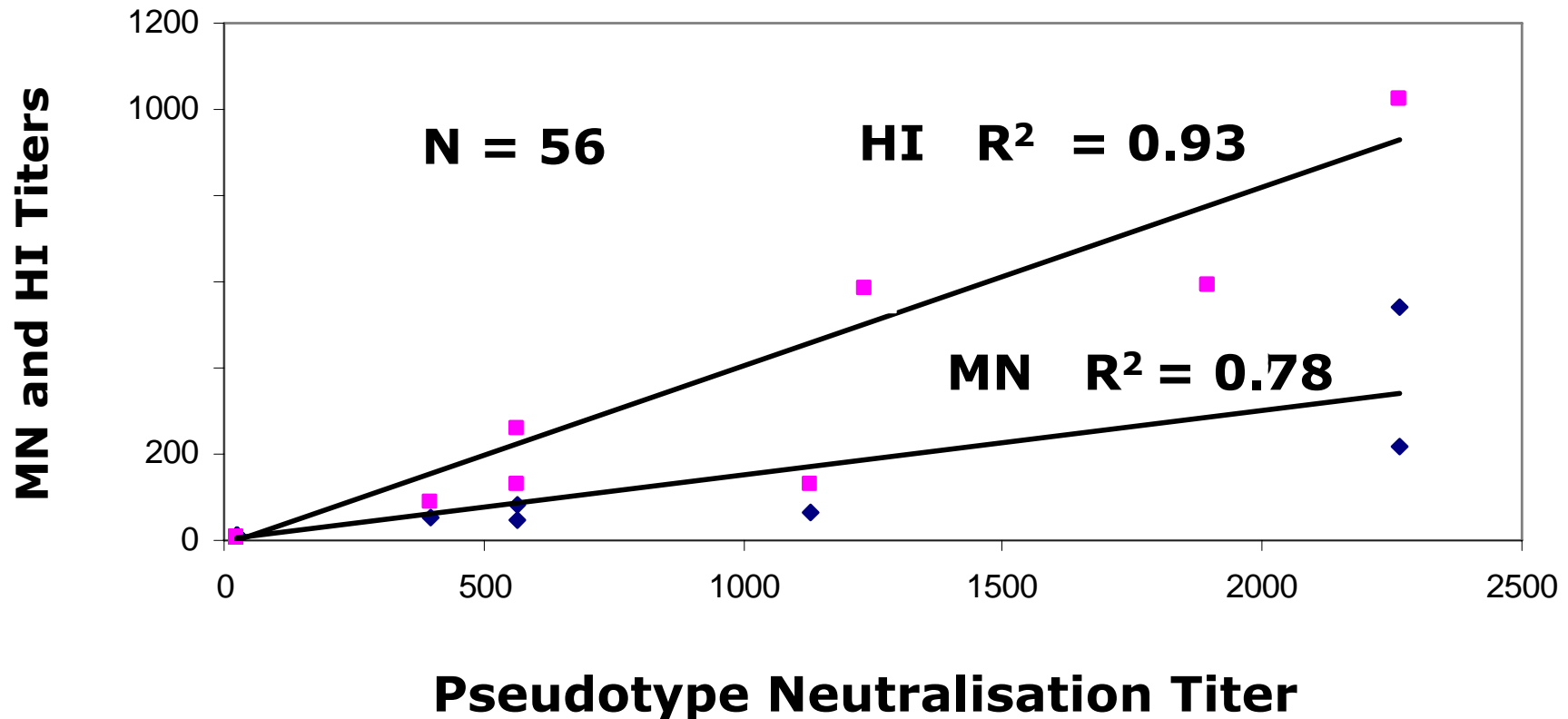


1:12,500



(Temperton *et al*, 2007)

H5N1 influenza: Pseudotype neutralisation correlates with hemagglutination inhibition



Nigel Temperton, Maria Zambon, Ian Brown, John Wood, M de Jong
UCL HPA VLA NIBSC Viet Nam
(Influenza & Other Respiratory Infections, 2007)

Combinations of HA H7 with NA N1 allow tests for "neutralisation" directed to NA of H5N1

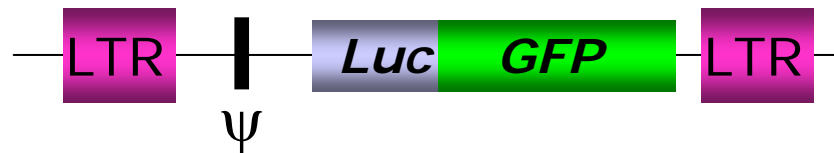
HIV



Hemagglutinin



Vector



+

Neurominidase



Findings on Neuramidase serology

Antibodies to NA are protective:

- **They neutralize enzyme activity**
- **They act on virus maturation not virus entry**
- **They do not neutralize virus particles**

Humoral immunity to enveloped viruses: we tend to forget complement

C1q



C3



MAC



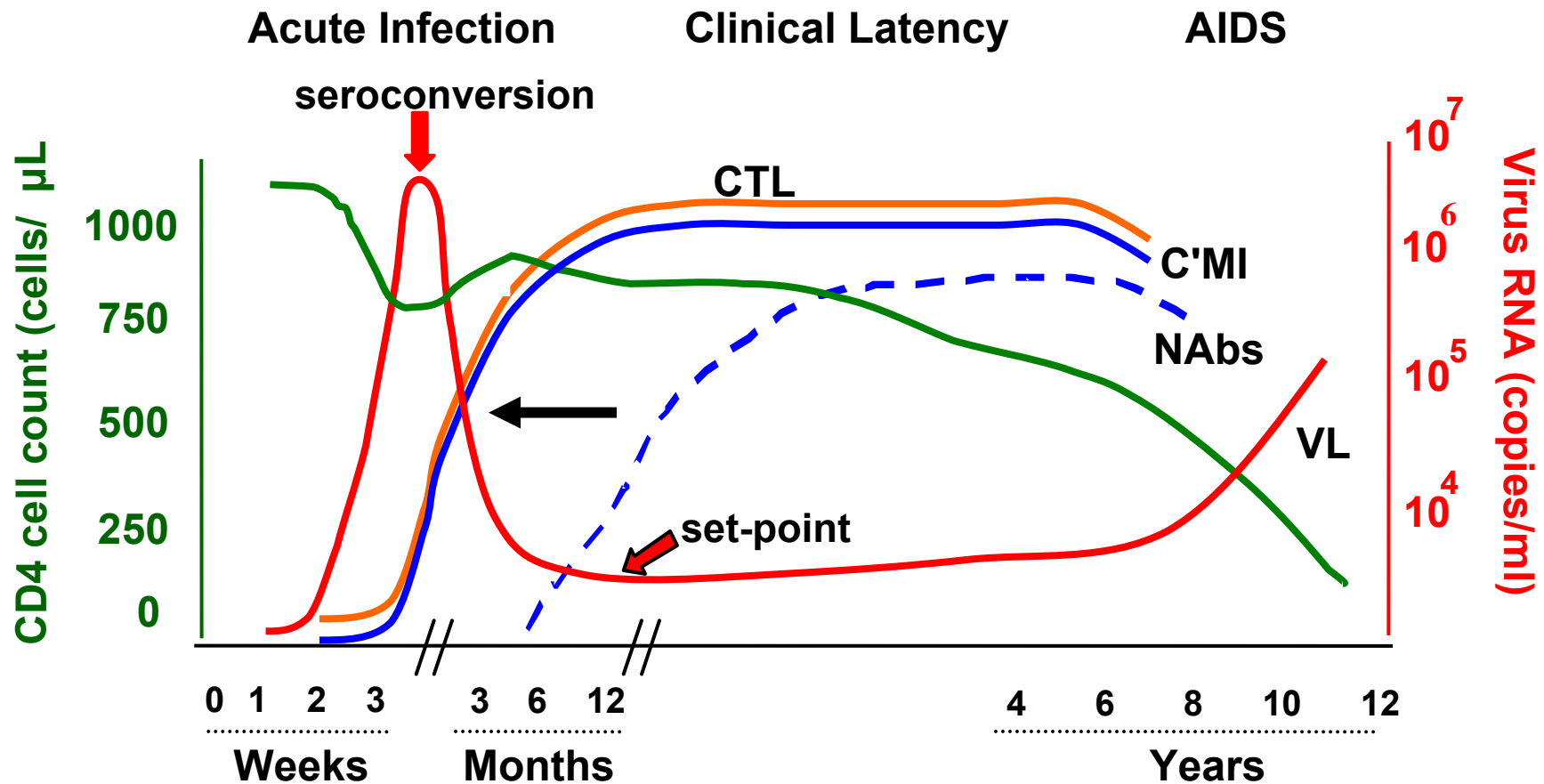
CRPs:

CD55

CD46

CD59

Development of Specific Immunity to HIV-1 Infection



(Aasa-Chapman et al., *J. Virol.* 2823-2830, 2005, A Trkola, PLoS Path, 2007)

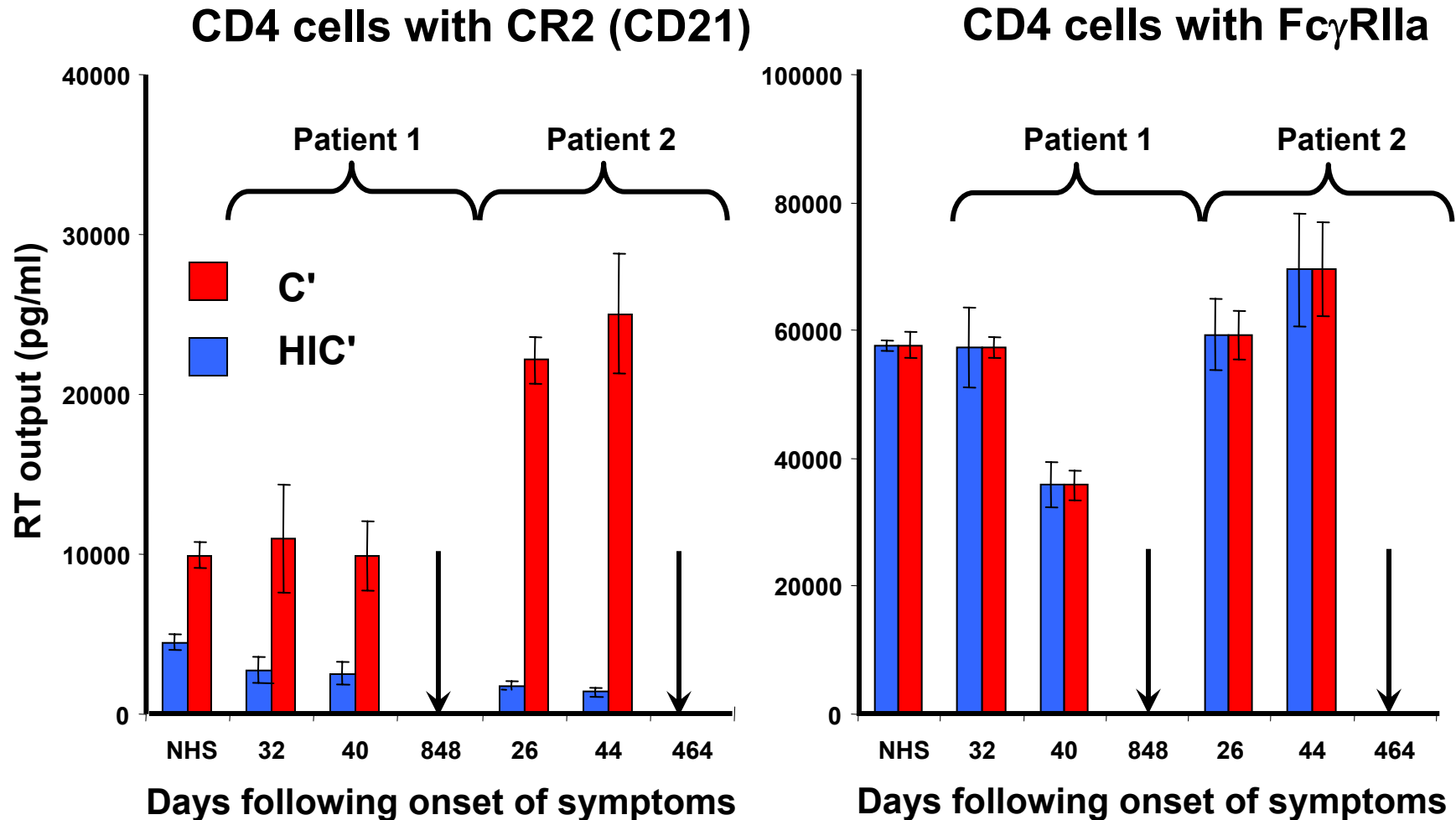
Non-neutralizing antibodies

- **May help to clear primary infection**
- **May be important in ADCC**
- **Denatured spikes and gp41 stumps exceed
number of functional spikes on HIV-1 virions**
- **May enhance infection**

Antibody Enhancement

- **Low affinity, poorly neutralizing Ab attach to virions**
- **Tethers virus to**
 - FcR**
 - Complement R**
- **Enhances virus infection of cells bearing FcR or CR, eg MØ**
- **Explains greater virulence of Dengue hemorrhagic fever**
 - upon second infection by a different serotype**
- **Could a similar problem arise for HIV based vaccines?**

How is antibody enhancement mediated in primary HIV infection?



(Suzy Willey, Marlen Aasa-Chapman, Aine McKnight, Robin Weiss, 2007)

Neutralizing antibody:

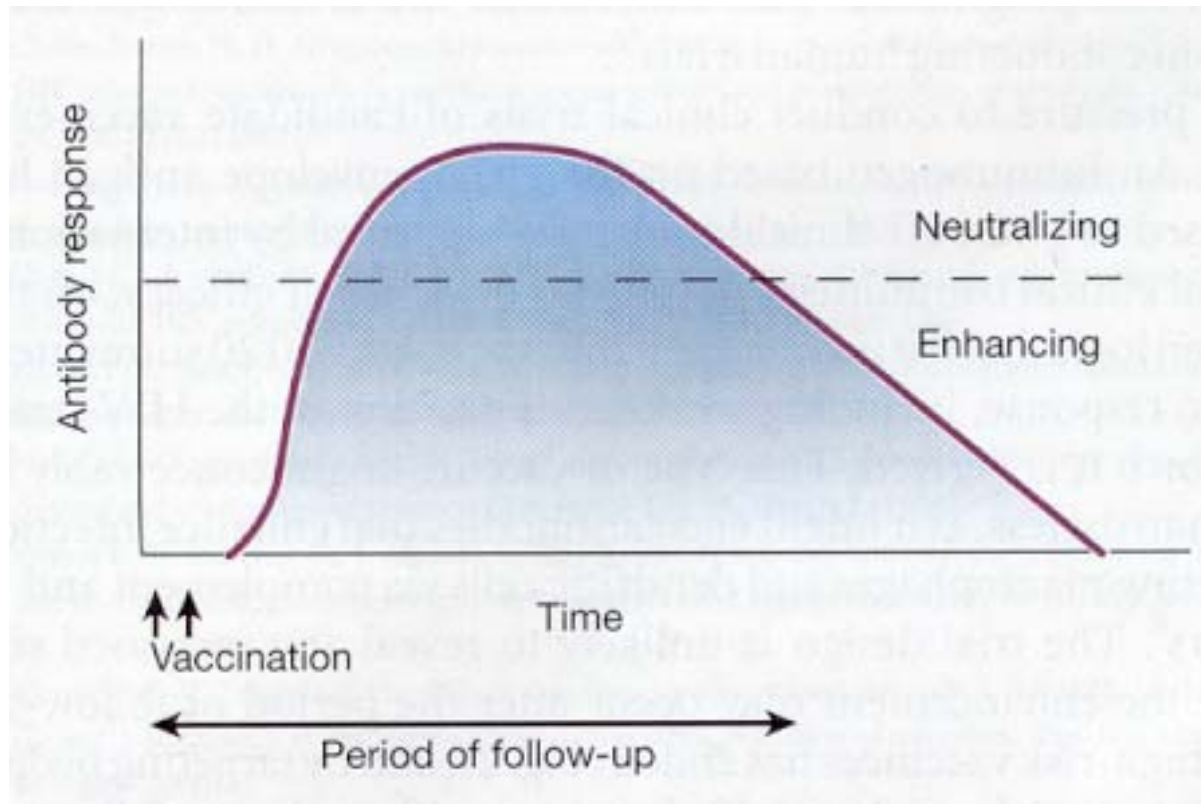
b12 (IgG1) more potent *in vivo* if Fc is intact (Dennis Burton)

Non-neutralizing antibody:

C' effector mechanisms may be more important

Could humoral immunity be harmful rather than protective?

(Weiss RA, Gulliver's travels in HIVland. *Nature* 410: 963 - 917, 2001)



**Vaccinees immunized with gp120:
greater enhancing titers than neutralizing titers
(Suzy Willey, thesis dissertation 2007)**

HIV diversity and neutralizing epitopes

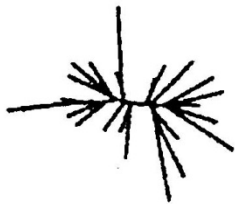
Global Influenza 1996



HIV Single Individual
6 Years Post Infection



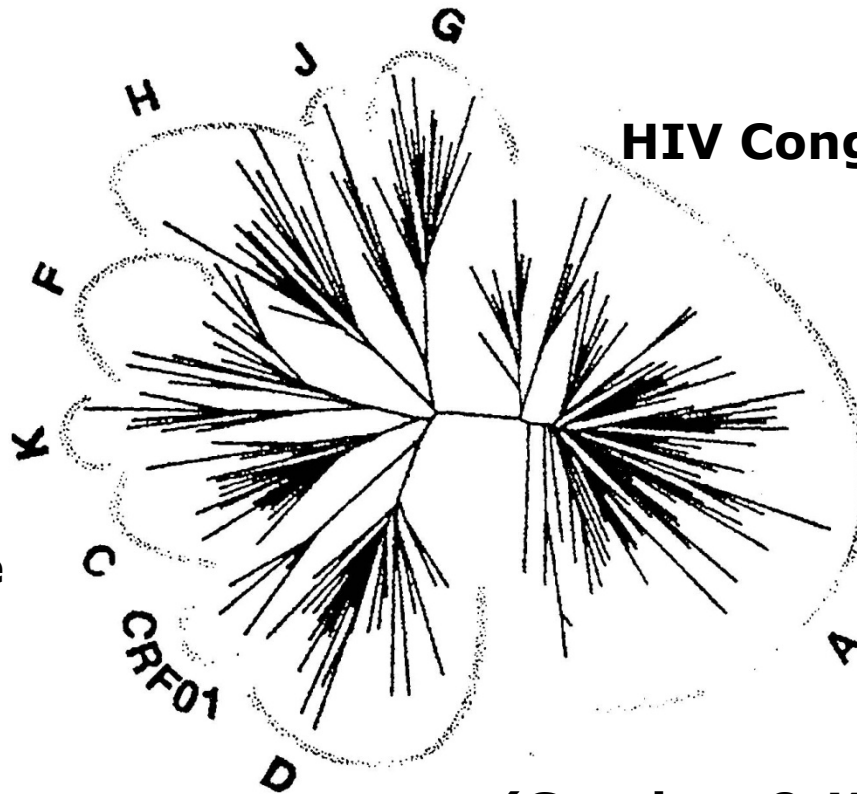
HIV
Amsterdam
Cohort 1991



The length of each spoke
indicates how far the
virus envelope has
mutated

.10

HIV Congo 1997



(Gaschen & Korber, 2001)

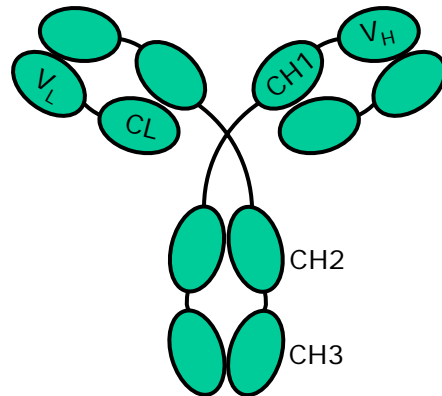
Llamas: a curious route to HIV neutralization



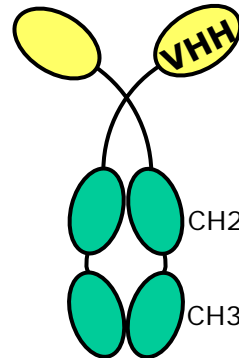
Hans de Haard & Theo Verrips
University of Utrecht



***Camelidae* (llamas, dromedaries, camels)
have classical and non-classical IgG**



Conventional antibody



Heavy-chain antibody



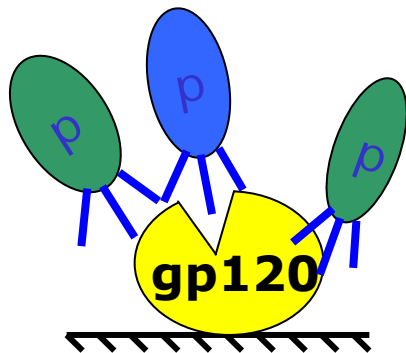
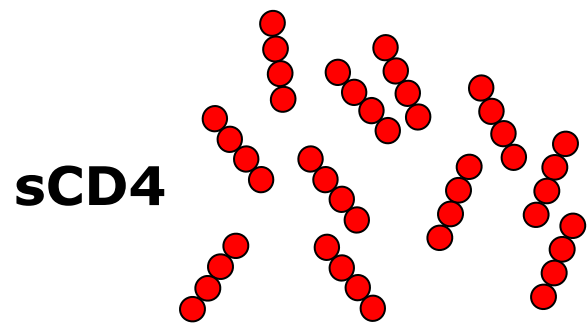
VHH

VHH recognising HIV may be useful as vaginal microbicides and for vaccine development:

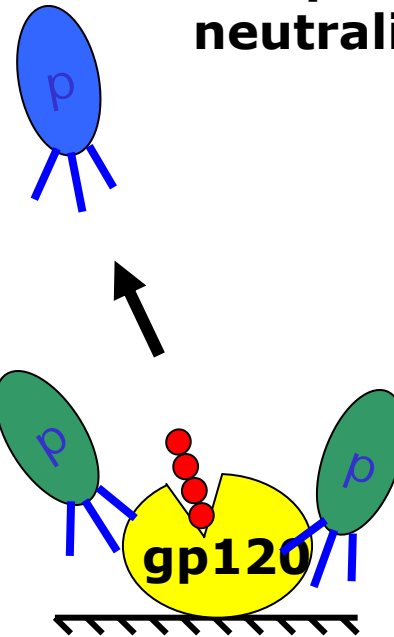
- **rapid screening of libraries**
- **long CDR3 loops**
- **small and stable 12-15kd proteins**
- **mapping neutralizing epitopes**

Screening for VHH targeting the CD4 binding site of gp120

Selection strategy: elution with soluble CD4



Express eluted VHH
Test: binding to gp120
competition with sCD4
competition with b12
neutralization of HIV-1



VHH neutralisation of selected HIV-1 isolates of subtype B and C

IC₉₀ (μg/ml)

VHH	B				C				
	III B	SF162	SVPB6	4.10.3	92BR025C	27d	ZA97001C	C222	C261
A12	1.7	7.8	•	>1	4.8	0.4	0.3	0.2	0.03
D7	3	9.3	•	0.01	•	0.4	0.3	•	0.08
C8	7	•	•	0.4	13	3.5	3	9	•
b12	0.8	1.6	1.3	•	•	0.3	0.1	•	•

• >50 μg/ml

Vaccine Targets – Protective Antibodies

- Preventing infection

 - Hepatitis B

 - Measles

 - HIV prophylactic vaccine



Emil von Behring

- Preventing virulence

 - Anti-toxins: Tetanus, Diphtheria

 - Disease symptoms: Marek's disease

 - HIV therapeutic vaccine

- Preventing systemic infection

 - Polio (non-sterilizing but effective)

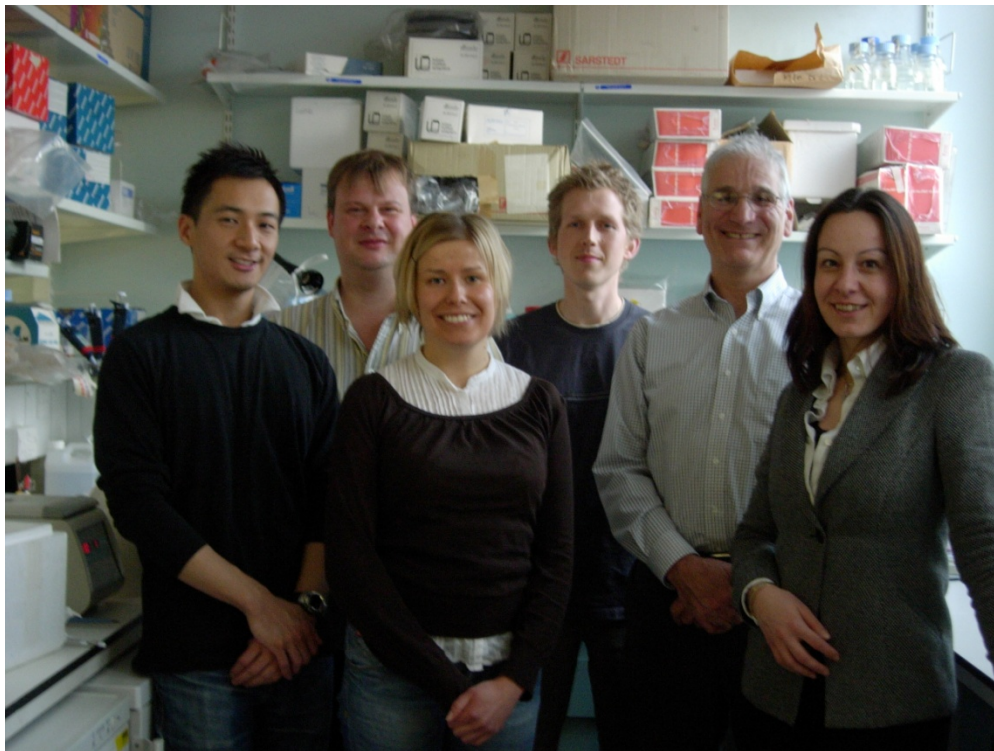
 - P Debré: protective, non-neutralizing HIV Ab

Conclusions:

Neutralization is important

**But there is more to protective humoral immunity
than neutralizing antibodies**

Beware enhancing antibodies in clinical trials



- Marlen Aasa-Chapman
- Anna Forsman
- Willie Koh
- Nigel Temperton
- Suzy Willey
- Ed Wright



BILL & MELINDA
GATES *foundation*

