



AMERICAN SOCIETY OF GENE & CELL THERAPY  
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## Presenter Disclosure

**Luk H. Vandenberghe, Ph.D.**

**The following relationships exist related to this presentation:**

**GlaxoSmithKline (GSK)  
ReGenX Biosciences**

**Inventor on licensed patents with the potential for  
royalties**

# B-cell Immunity to Viral Vectors

Luk H. Vandenberghe, Ph.D.



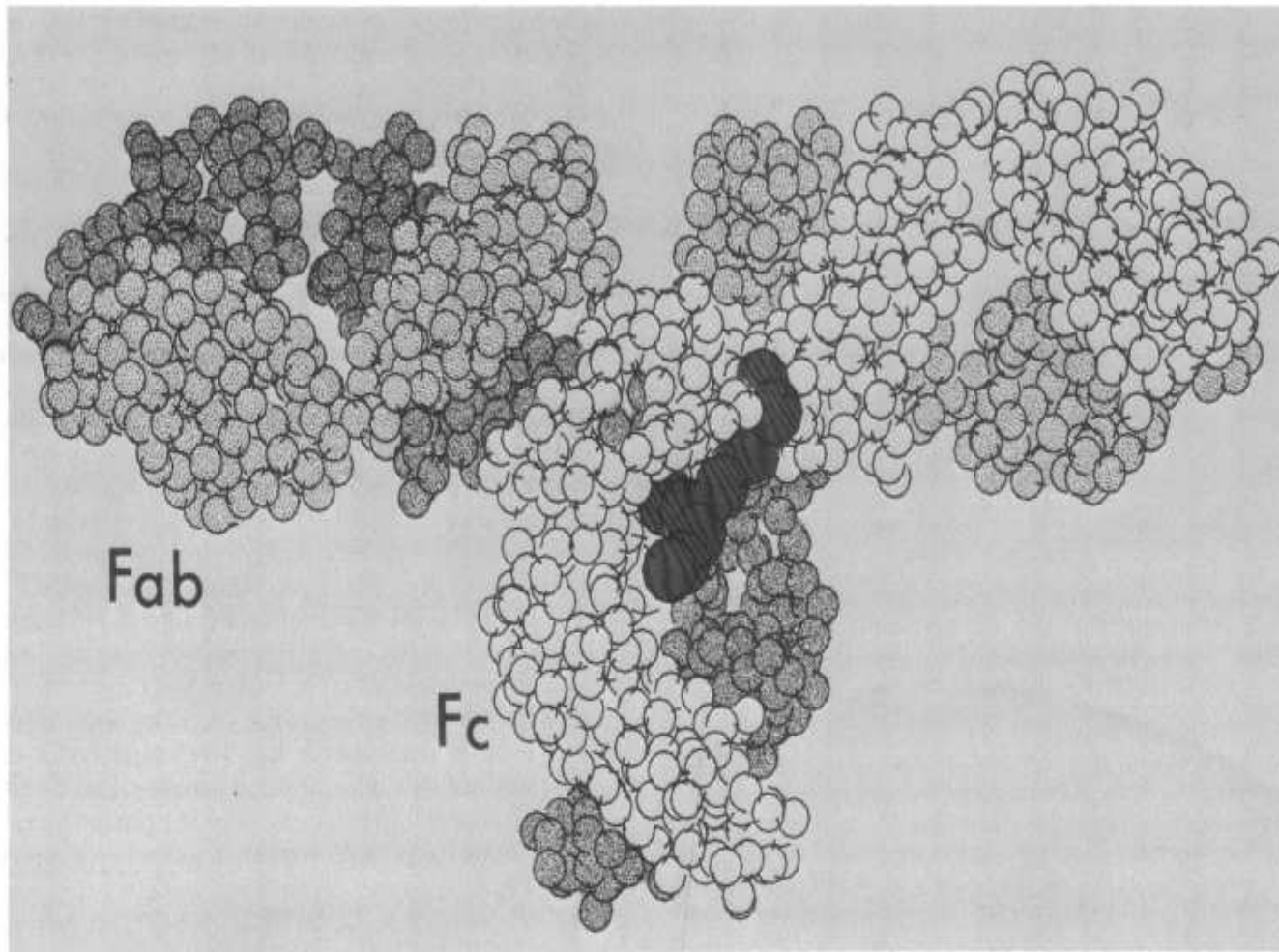


FIG. 3. Space-filling view of the Dob Ig molecule. One complete heavy chain is in white and the other is dark gray; the two light chains are lightly shaded. The large black spheres represent the individual hexose units of the complex carbohydrate. In this view, the 2-fold axis of symmetry is vertical. A crevasse is seen between the  $C_{H2}$  of the white heavy chain and the  $C_L$  domain of the Fab on the left.

## Three-dimensional structure of an intact human immunoglobulin

(antibodies/x-ray diffraction/glycoprotein/carbohydrate)

E. W. SILVERTON, MANUEL A. NAVIA, AND DAVID R. DAVIES

Laboratory of Molecular Biology, National Institute of Arthritis, Metabolism and Digestive Diseases, National Institutes of Health, Bethesda, Maryland 20014

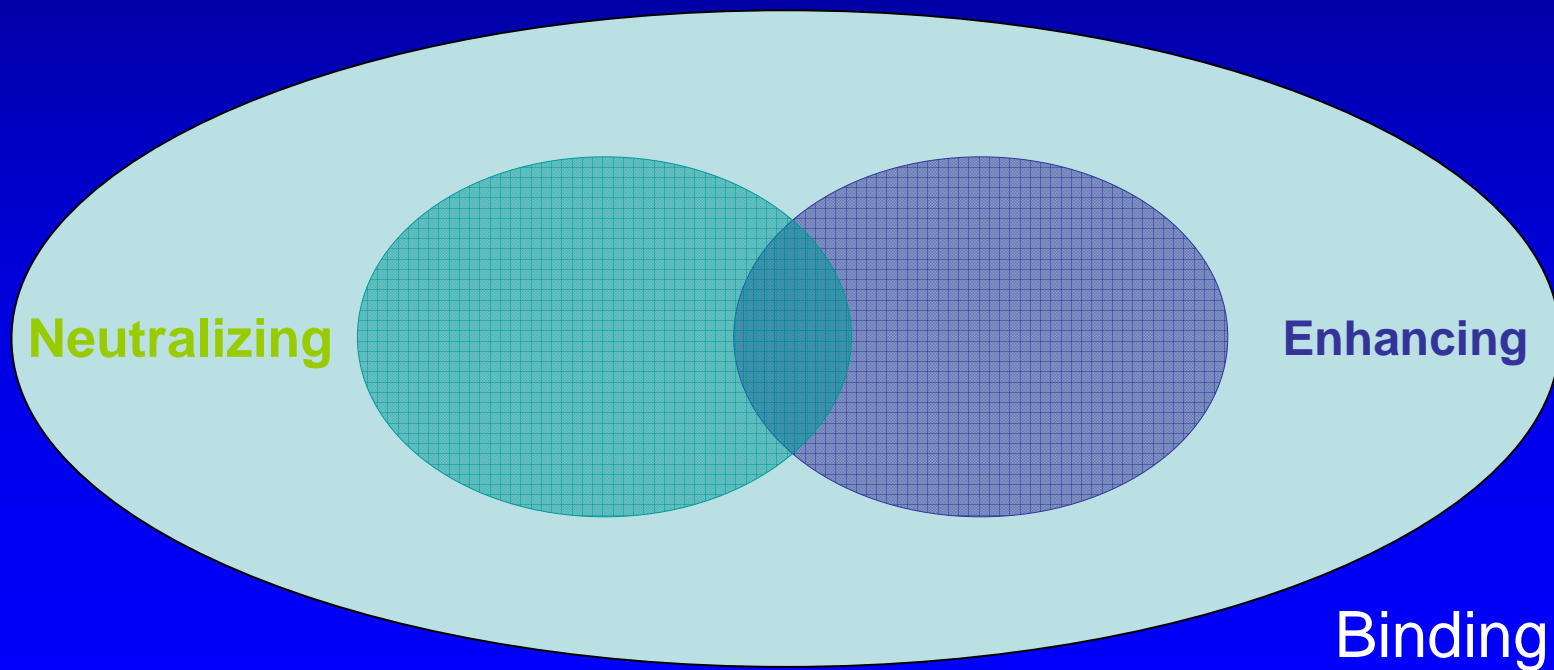
*Proc. Natl. Acad. Sci. USA*  
 Vol. 74, No. 11, pp. 5140-5144, November 1977  
 Immunology

# Neutralizing Antibodies

- 'Abrogation of virus (vector) infectivity by the binding of Abs to the particle'

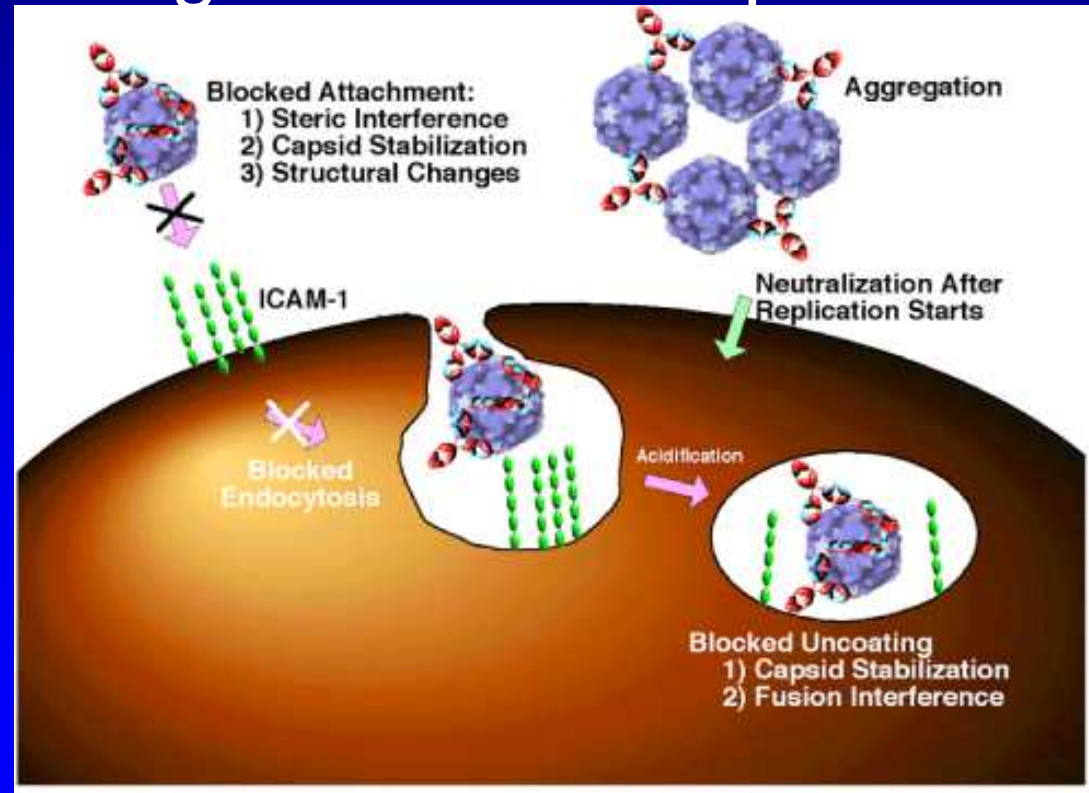
# Neutralizing Antibodies

- 'Abrogation of virus (vector) infectivity by the binding of Abs to the particle'



# Neutralizing Antibodies

- ‘Abrogation of virus (vector) infectivity by the binding of Abs to the particle’



Human Rhinovirus, Smith TJ, 2002

# AAV8 GFP expression in NHP liver (d7)

NAB  
AAV8

1/10

605045 middle 10x

605045 right 10x

605045 right 20x

<1/5

607313 middle 10x

607313 left 10x

607313 middle 20x

<1/5

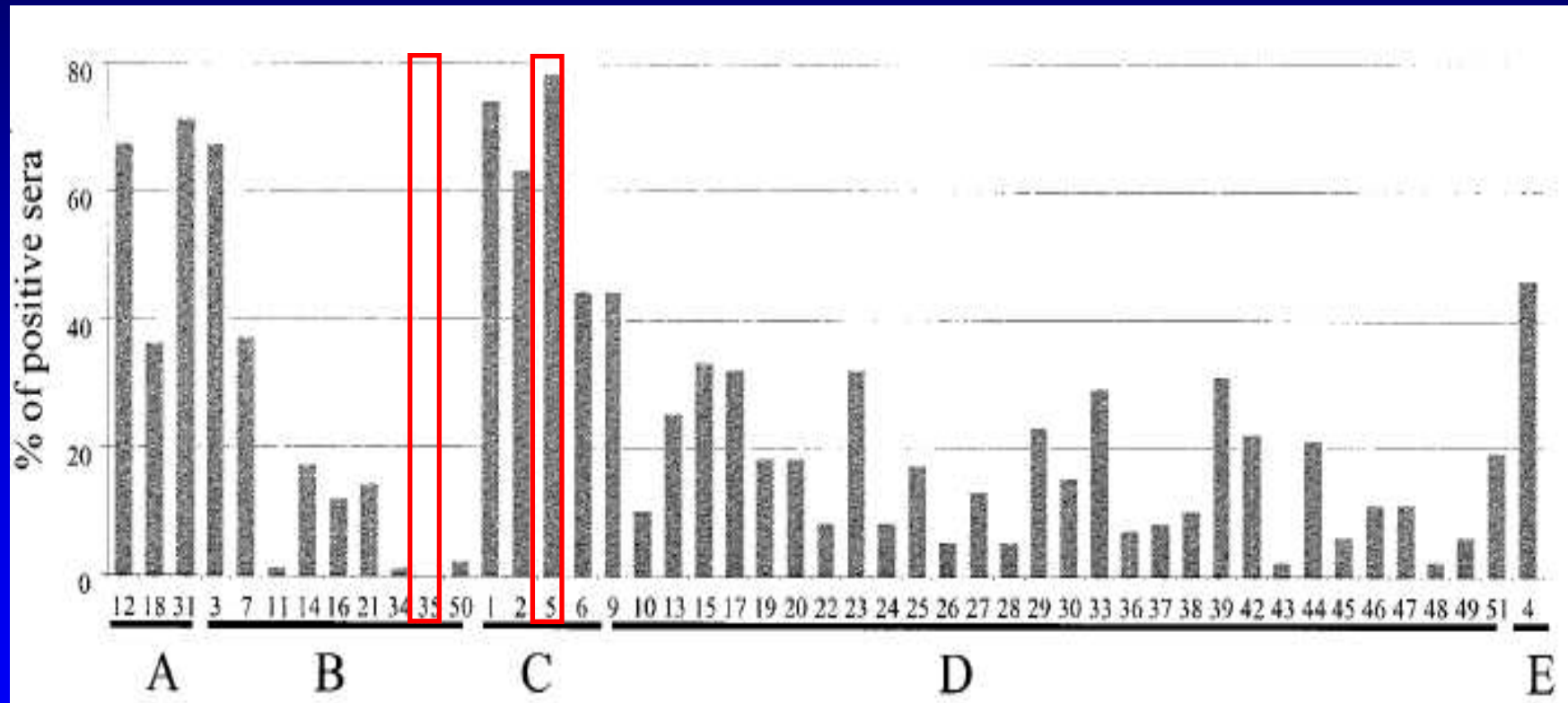
608059 middle 10x

608059 left 10x

608059 middle 20x

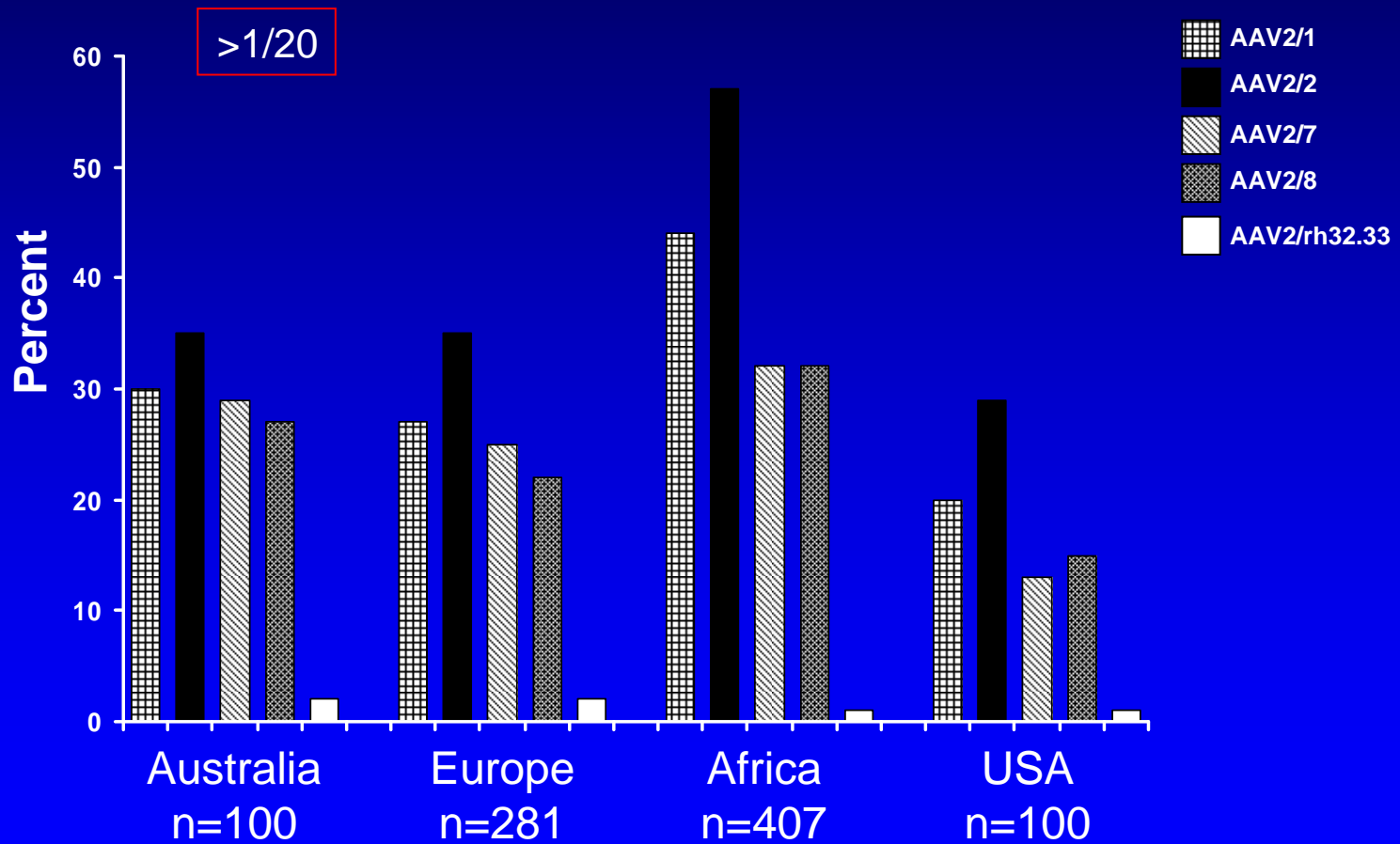
Wang et al, MT, 2010

# Seroprevalence due to Natural Infection *Adenovirus*



Vogels et al. JVI 2003

# Seroprevalence due to Natural Infection *Adeno-associated Virus (AAV)*

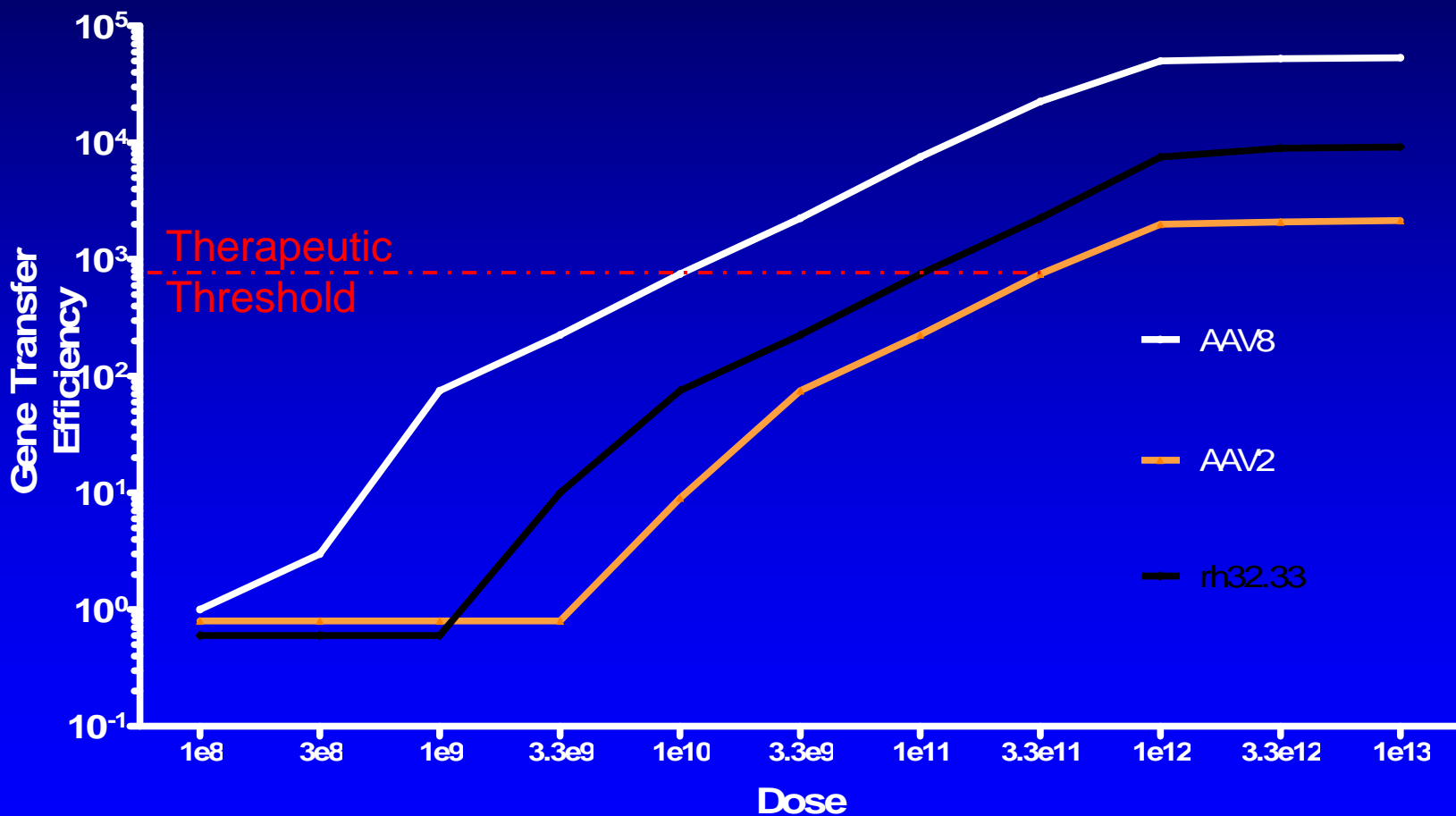


# Substantial AAV2 Neutralizing Capacity Of Human Plasma

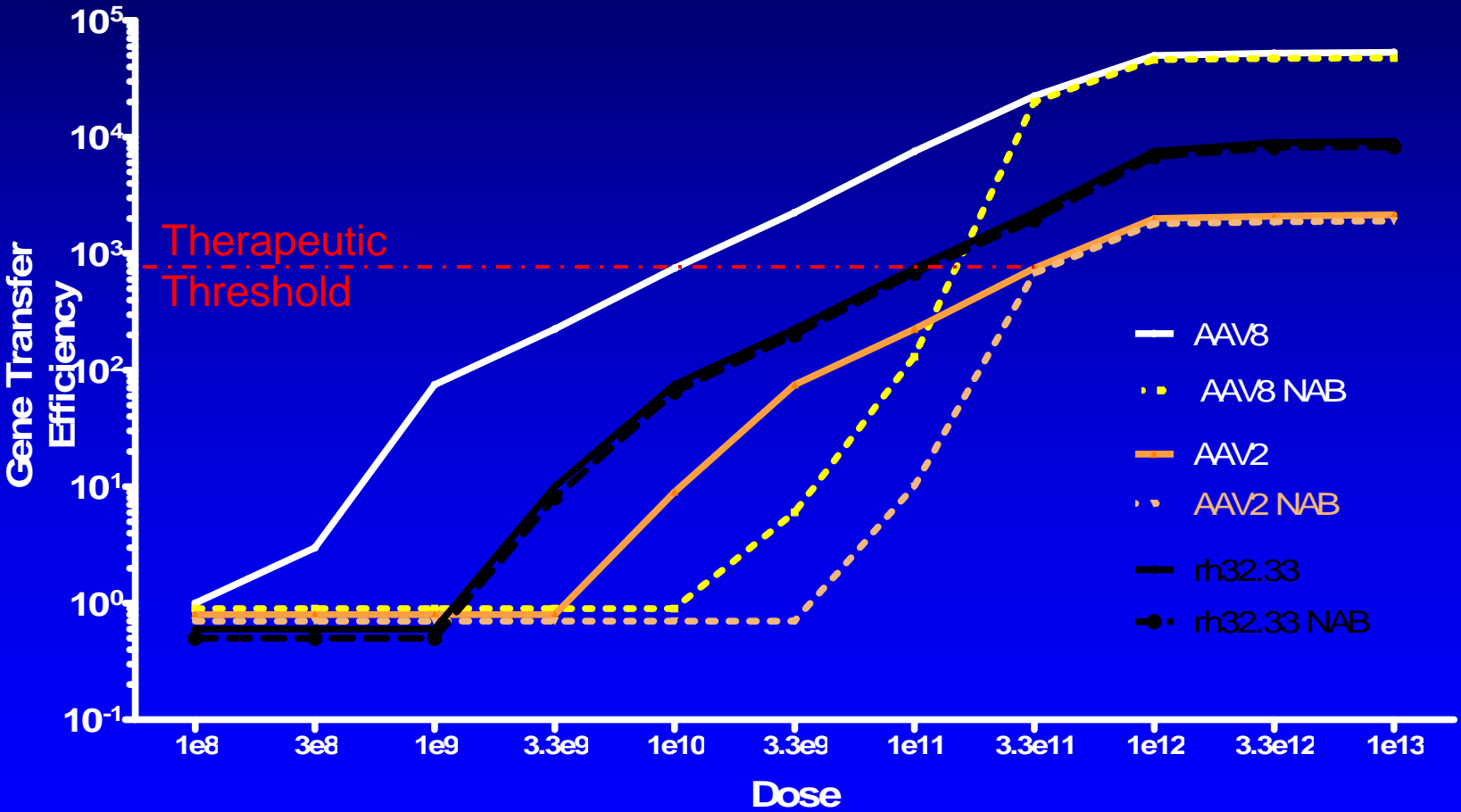
Neutralizing Titer	Volume		
	15 $\mu$ L	1mL	1L
0	$< 8 \times 10^9$	$< 5 \times 10^{11}$	$< 5 \times 10^{14}$
2	$8 \times 10^9 - 8 \times 10^{10}$	$5 \times 10^{11} - 5 \times 10^{12}$	$5 \times 10^{14} - 5 \times 10^{15}$
20	$8 \times 10^{10} - 3.6 \times 10^{11}$	$5 \times 10^{12} - 2 \times 10^{13}$	$5 \times 10^{15} - 2 \times 10^{16}$
200	$> 3.6 \times 10^{11}$	$> 2 \times 10^{13}$	$> 2 \times 10^{16}$

Courtesy of P. Colosi/Avigen – currently NEI

# Model of Pharmacodynamics of Improved Vectors in the presence of NAB



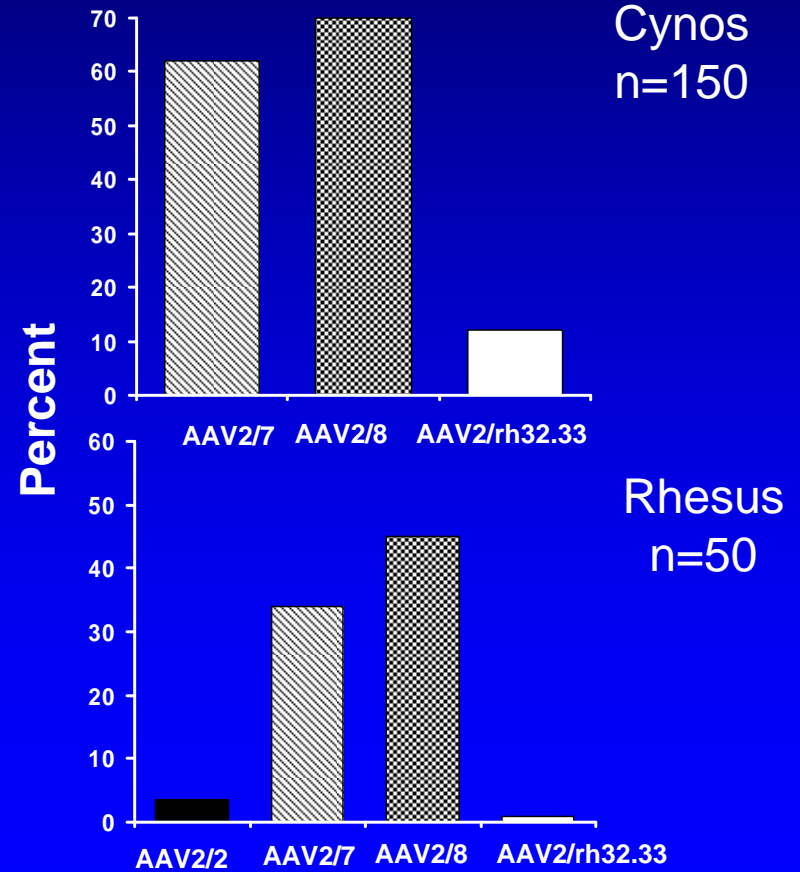
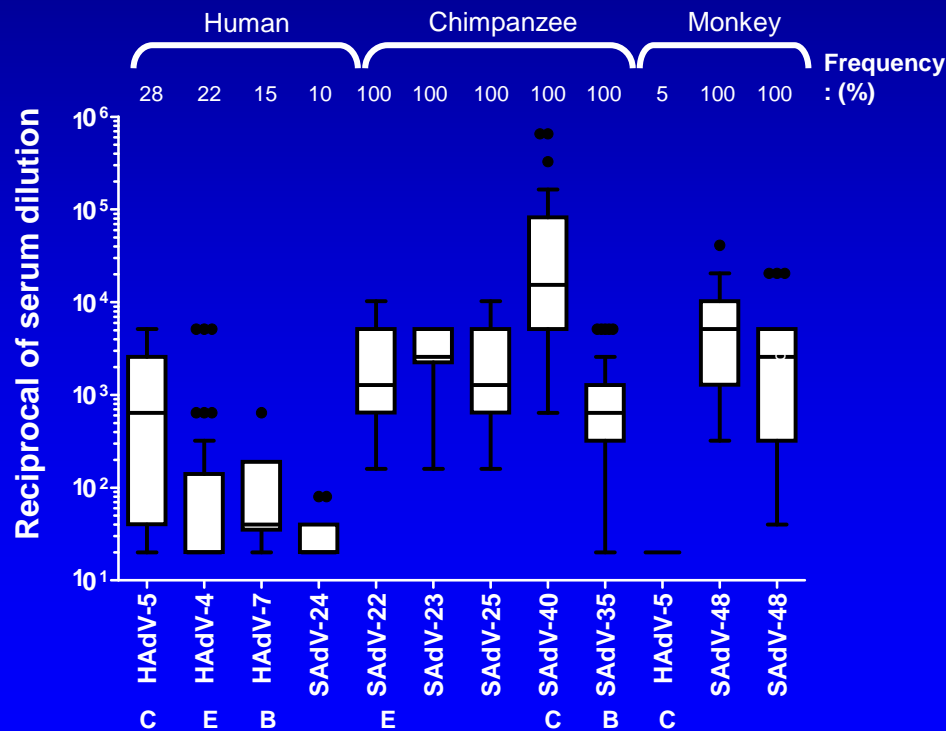
# Model of Pharmacodynamics of Improved Vectors in the presence of NAB



# Serological prevalence in preclinical primate models as well...

## Ad

## AAV

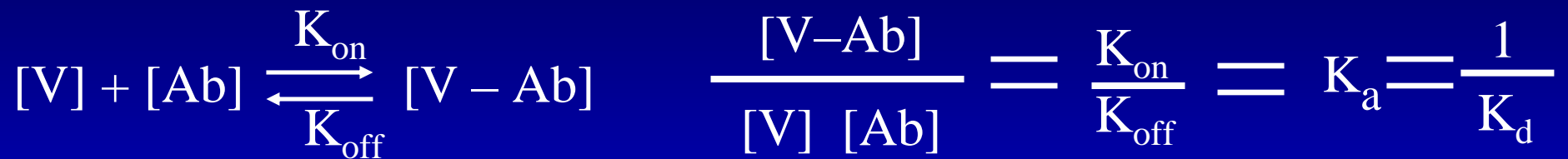


# Antibody-virus binding

## “Percentage Law”

- Over a wide range of virion concentrations, a given concentration of antibody neutralizes the same relative proportion of virus infectivity
  - Andrewes, C.H. & Elford, W.J. Observations on anti-phage sera I. ‘The percentage law’. Br. J. Exp. Path. (1933)

# Antibody-virus binding “Percentage Law”



At (50% neutralization) free virus conc. [V]  
equals bound virus conc. [V-Ab].

At this point the antibody concentration [Ab]  
is dependent on affinity, not on virus concentration

*if antibody is in excess, i.e.,  
virus binding does not change antibody concentration*

**\*Exception: Hit & Run Virus Neutralization**

# Antibody-virus binding

## “Percentage Law” – some back of the envelope calculations

- Serum IgG conc – 1.1 g/dl = 11mg/ml
- *If 0.1% is neutralization specific, 11μg/ml*
- 11μg/ml = 73 nM ( $73 \times 10^{-9}$  M) = approx.  $4 \times 10^{13}$  molecules IgG/ml
- If  $K_d$  = approx. 1 nM, 50% virus will be neutralized at 73 x dilution (1/73 NAB titer)
- 100 μl of a 100 x dilution contains  $4 \times 10^{10}$  molecules IgG

# Poliovirus neutralization and the Percentage Law

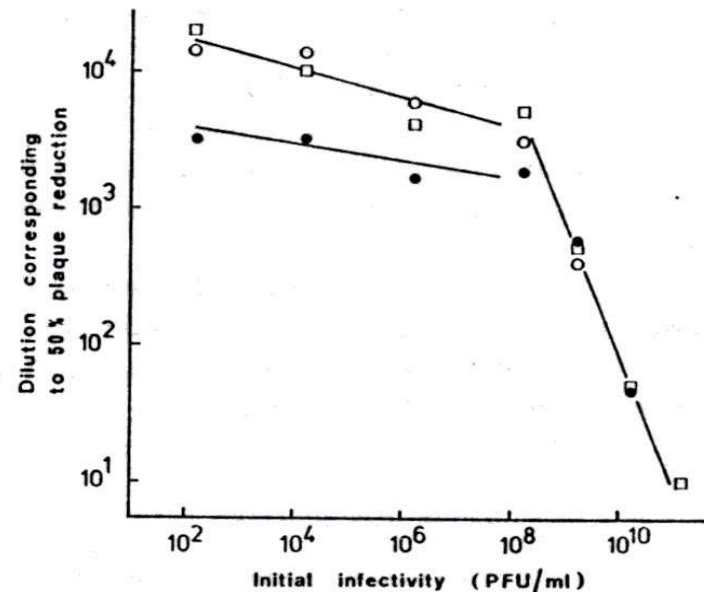


Fig. 2. Effect of the amount of virus on the dilution of antibodies ensuring 50 per cent neutralization. Data were taken from Table 1, last column. Open circles, polyclonal serum; filled circles, MoAb 36-5h2; squares, MoAb 35-1f4

*“The degree of neutralization by mono- and polyclonal antibodies is determined by the antibody/virion ratio at virus concentrations exceeding  $10^8$  PFU/ml, and by antibody concentration alone at lower doses.”*

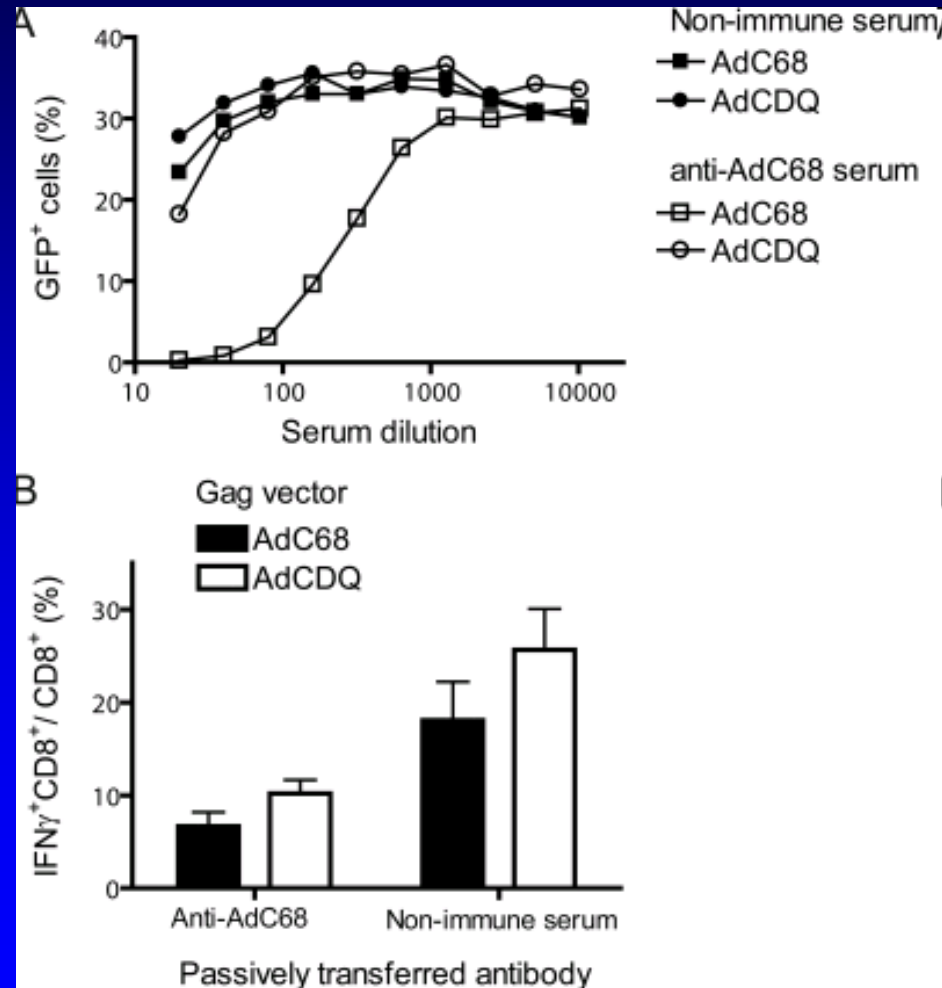
*Brioen, P. & Boeye, A. Arch. Virol. 83, 105 (1985)*

# Detection

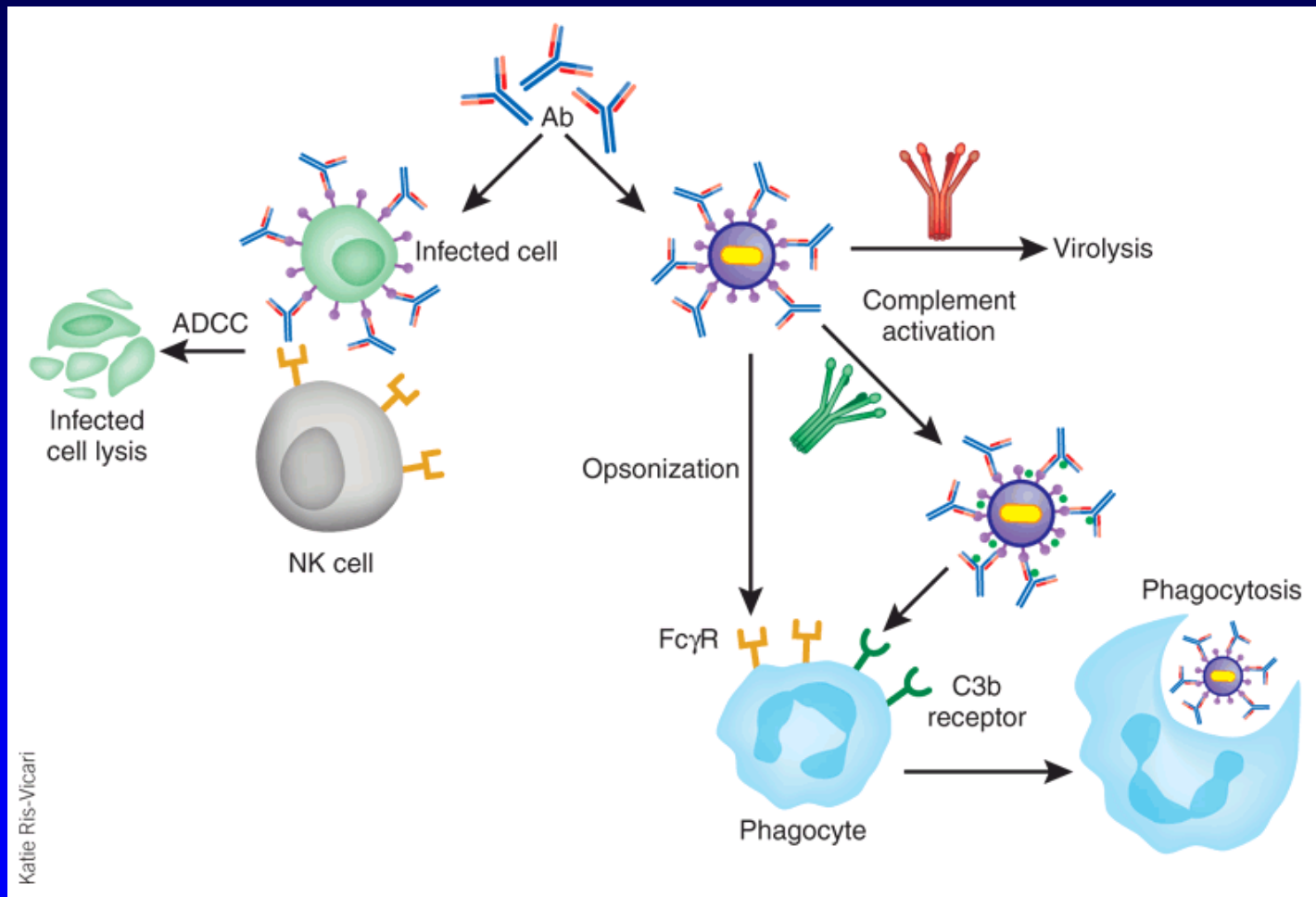
## Parameters for design of NAB assay

- *In vitro* NAB assay
  - $<10^9$  pt/mL
  - Normalize by physical, not infectious titer
  - Titer:
    - Dilution at which XX% neutralization is achieved
- Passive transfer *In vivo* neutralization
  - Vector dose /  $t_{1/2}$
  - Dose of Ig / Blood volume
  - Non-Neutralizing effects

# Limitations of *in vitro* NAB



# Non-Neutralizing effects of Ab

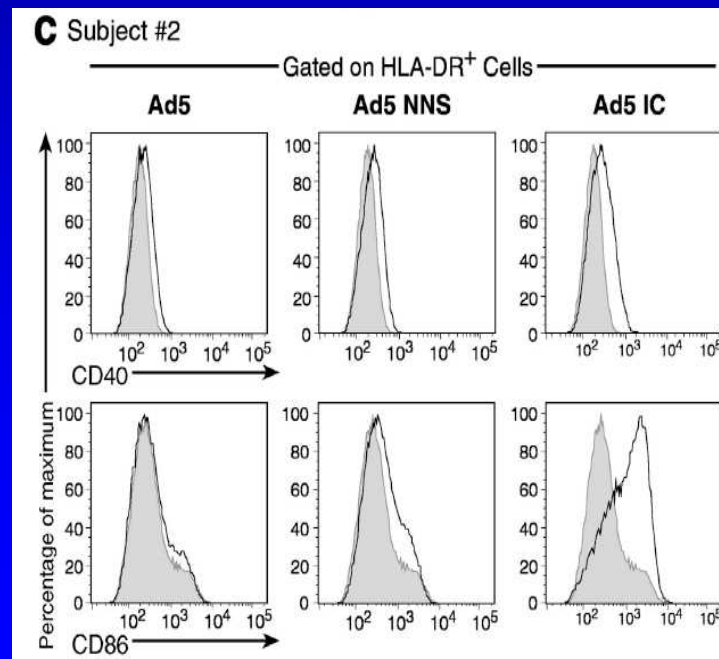


Marasco & Sui, Nature Biotechnology 25, 1421 - 1434 (2007)

# NAB, Immune-activation and STEP

<u>Ad5 Titer (1/x)</u>	<u>18</u>	<u>18-200</u>	<u>200-1000</u>	<u>&gt;1000</u>
Vaccine	20	8	14	7
Placebo	20	4	7	2

Buchbinder et al., Lancet 2008



Perreau et al., JCI 2009

# Breadth of NAB Response and Cross Reactivity

Vector pseudotypes used in the neutralization assay									
from rabbit	AAV2/1	AAV2/2	AAV2/3	AAV2/4	AAV2/5	AAV2/6	AAV2/7	AAV2/8	AAV2/9
immunized with:									
AAV2/1	1/163,840	No NAB	No NAB	No NAB	1/40,960	1/40,960	1/40	No NAB	No NAB
AAV2/2	1/80	1/163,840	1/5,120	1/20	No NAB	1/80	1/40	1/40	No NAB
AAV2/3	1/1,280	1/2,560	1/40,960	1/20	1/40	1/2,560	1/1,280	1/1,280	No NAB
AAV2/4	1/20	No NAB	No NAB	1/1,280	1/40	No NAB	No NAB	No NAB	1/40
AAV2/5	1/20,480	No NAB	1/80	No NAB	1/163,840	1/5,120	1/40	No NAB	No NAB
AAV2/6	1/81,920	No NAB	1/640	1/40	1/40	1/327,680	1/40	No NAB	1/40
AAV2/7	1/1,280	1/640	1/1,280	1/20	No NAB	1/1,280	1/163,840	1/5,120	1/80
AAV2/8	1/20	1/1,280	1/1,280	No NAB	1/20	No NAB	1/640	1/327,680	1/2,560
AAV2/9	No NAB	No NAB	No NAB	No NAB	No NAB	No NAB	1/20	1/640	1/20,480

homologous

> 1/16 homol.

1/16 homol.

< 1/16 homol.

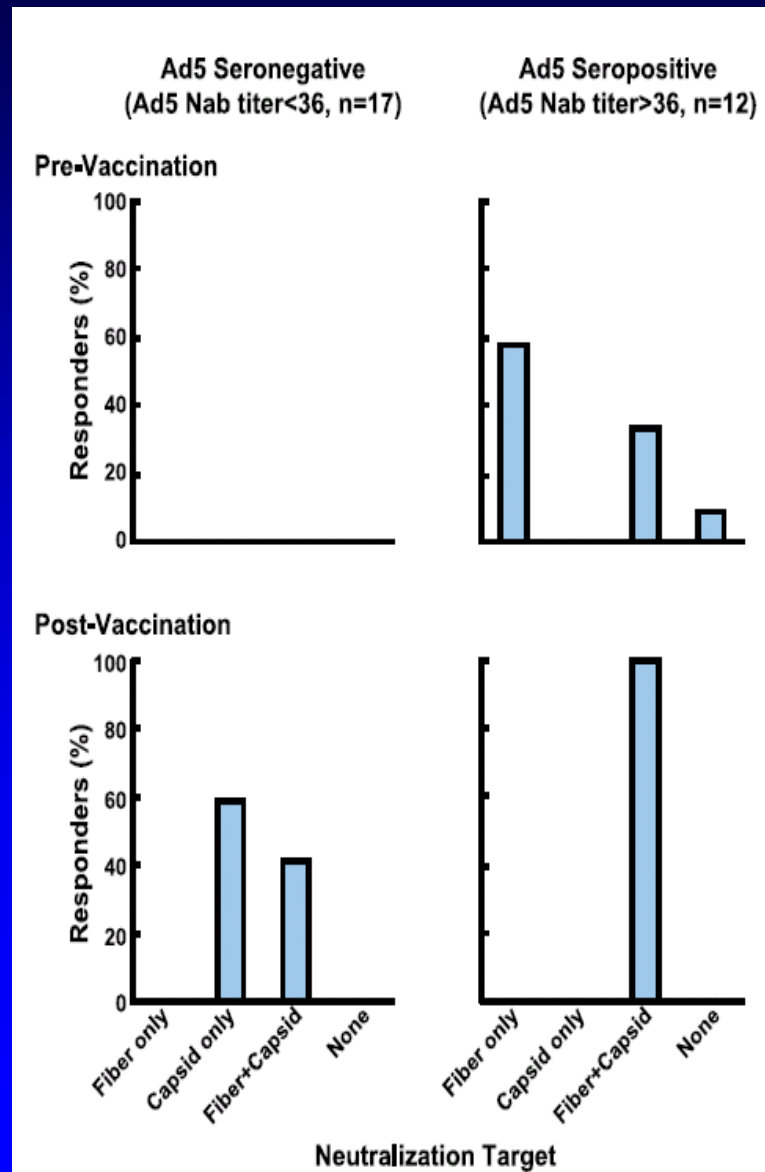
No NAB

# Likelihood of cross reactivity in human sera

**Table 2. Linkage of seroreactivity between adeno-associated virus (AAV) serotypes in humans from 4 geographic areas.**

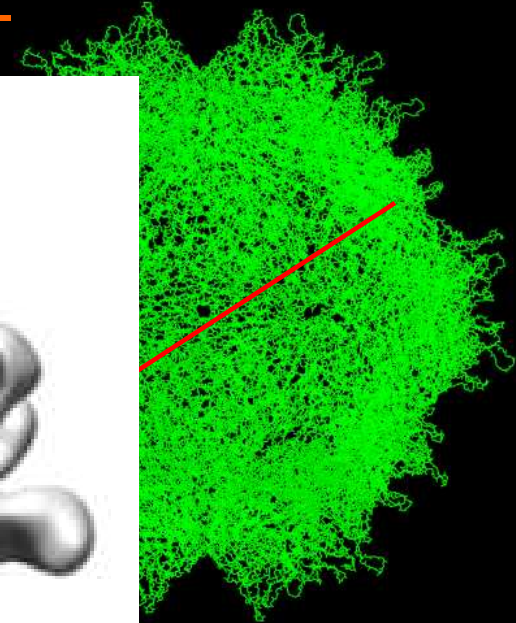
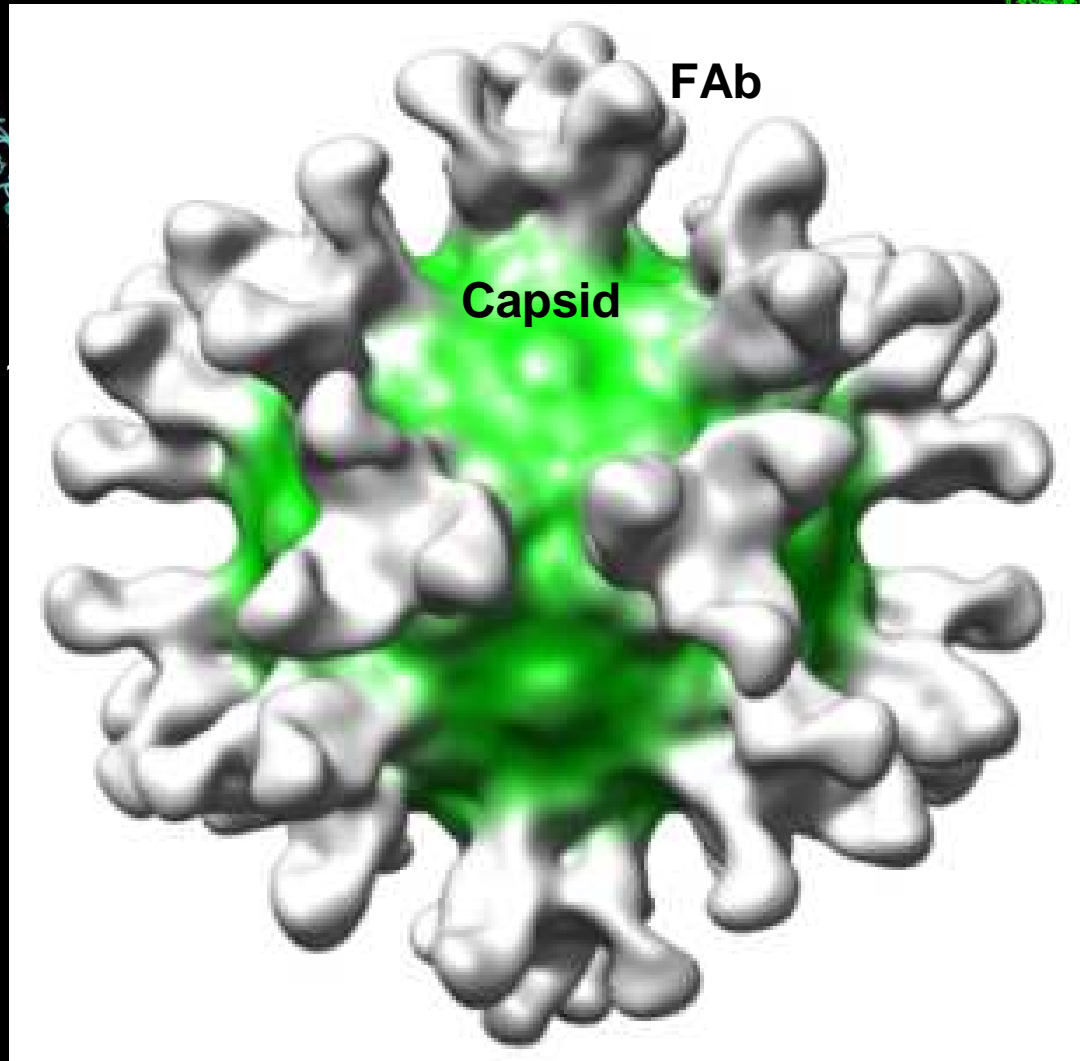
NAB titer, region, seroprevalent serotype	Seroreactivity linkage ratio			
	AAV1 <sup>+</sup> /AAV1 <sup>-</sup>	AAV2 <sup>+</sup> /AAV2 <sup>-</sup>	AAV7 <sup>+</sup> /AAV7 <sup>-</sup>	AAVB <sup>+</sup> /AAVB <sup>-</sup>
<b>&gt;1:20</b>				
<b>Africa</b>				
AAV1	---	3.5	4.5	4.0
AAV2	2.2	---	2.2	1.8
AAV7	17.2	9.7	---	6.4
AAVB	10.7	5.7	14.8	---
<b>Europe</b>				
AAV1	---	26.3	29.4	11.5
AAV2	7.0	---	6.8	5.0
AAV7	184.5	126.2	---	17.3
AAVB	51.8	35.3	57.9	---
<b>Australia</b>				
AAV1	---	26.0	9.8	8.9
AAV2	9.3	---	5.3	6.8
AAV7	11.2	8.9	---	10.4
AAVB	13.4	11.6	14.1	---
<b>United States</b>				
AAV1	---	1.3	1.2	0.3
AAV2	1.3	---	1.1	3.0
AAV7	1.2	1.0	---	12.8
AAVB	0.3	1.7	4.3	---

# Breadth – Natural Infection vs Vaccination



# 3-D Analysis of AAV:FAb Complexes

FAb  
Crystal structure

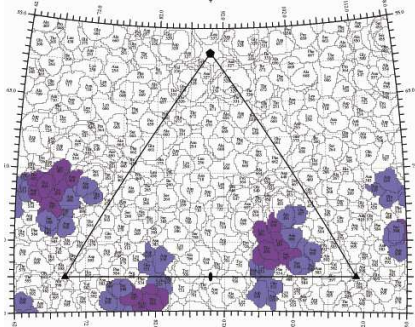
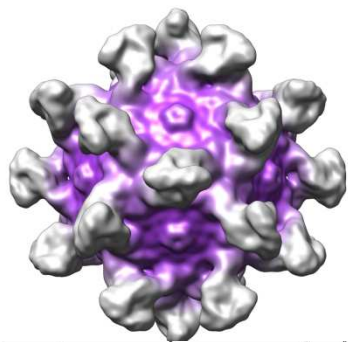


al structures ~2.5 -3.5  
Å

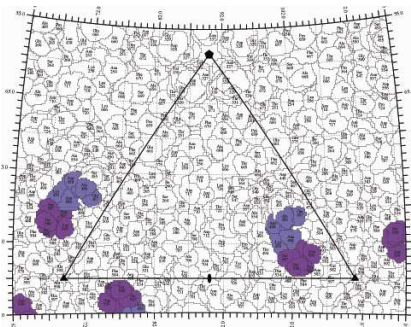
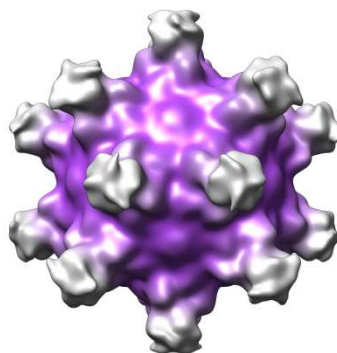
Capsid: FAb  
complex  
structure  
by Cryo-EM

(Gurda and McKenna, in preparation)

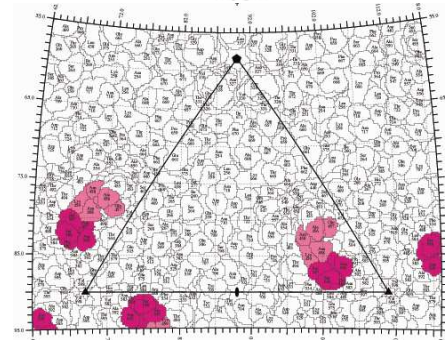
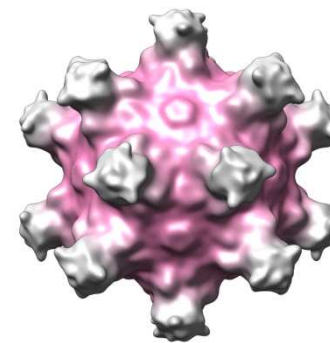
AAV1:  
G7



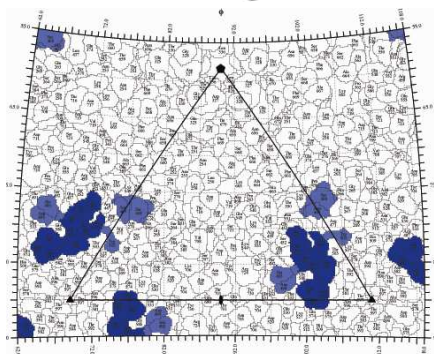
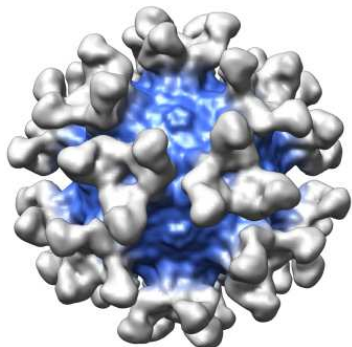
AAV1:  
D11



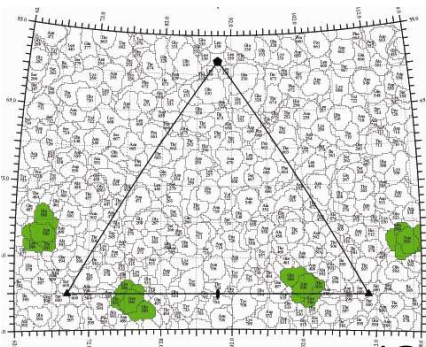
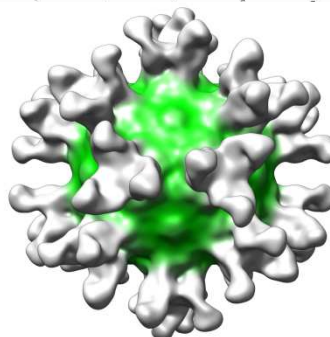
AAV6:  
D11



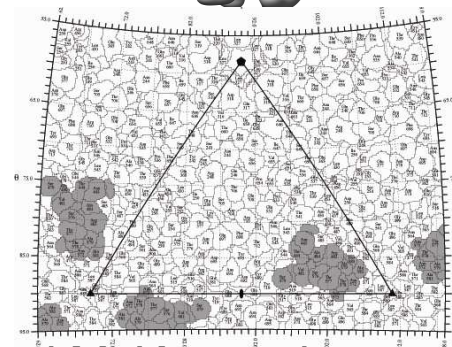
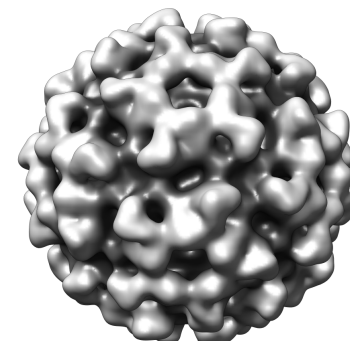
AAV2:  
C37B



AAV8:  
ADK8



AAV5:F4



(Gurda and McKenna, in preparation)

# Circumventing NAB

- Anatomical
- Route of Administration
- Surgical
  
- Immune Suppression
  
- Chemical modification
- Serotype switching
- Vector Engineering

# Acknowledgments

Soumitra Roy

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Peter Colosi

James M. Wilson