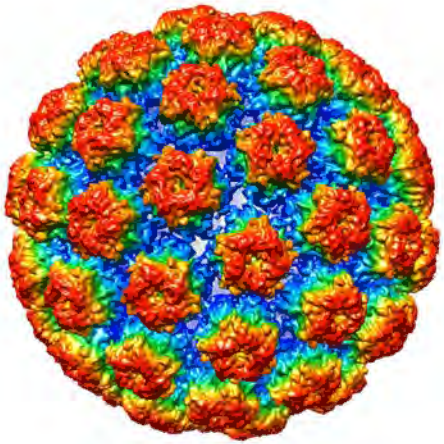
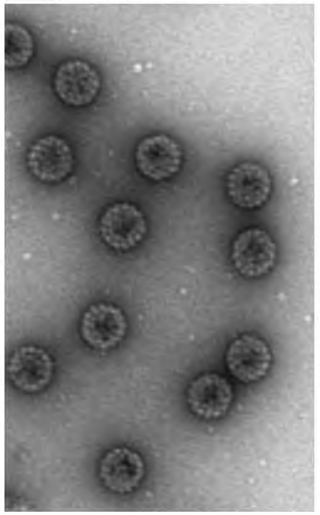
Why the HPV VLP Vaccines Are So Effective

John Schiller
Center for Cancer Research
National Cancer Institute, NIH



It's Multifactorial

- HPVs have DNA genomes so can't evolve rapidly to evade nAb responses.
- The vaccines are exceptionally good at inducing neutralizing antibodies.
- Infection mechanism make HPVs exceptionally susceptible to neutralizing antibodies.



HPV16 L1 VLPs

Provides plausibility for HPV VLPs as the first subunit vaccine to induce long term protection after a single dose

Consistency of Seroconvertion to VLPs

Percent of Girls Seroconverting to Cervirix or Gardasil-9*

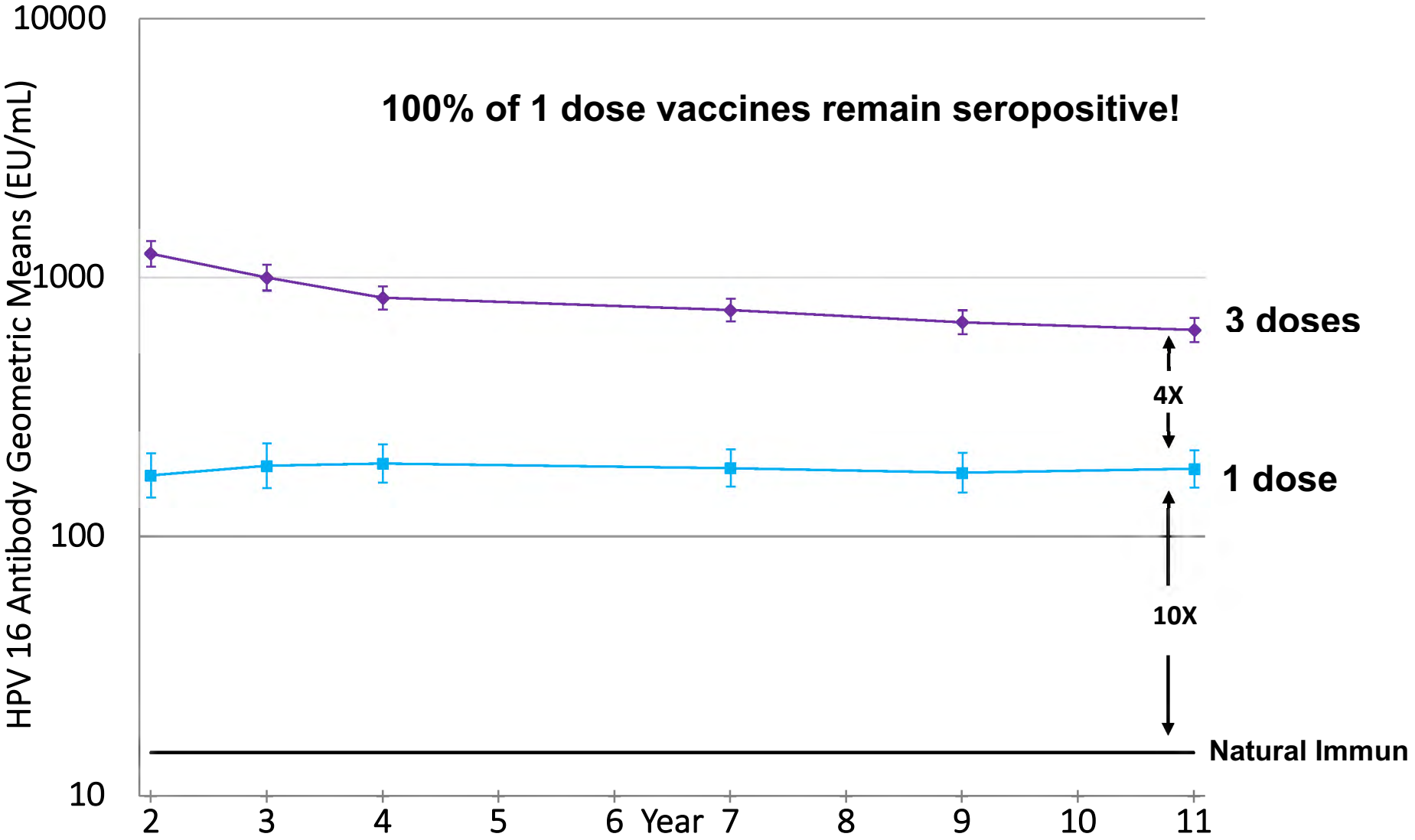
Doses	Cervarix		Gardasil-9	
	HPV16	HPV18	HPV16	HPV18
One	99.3%	98.6%	100%	98.5%
Two	100%	100%	100%	100%
Three	99.3%	99.3%	100%	100%

- One Months after last dose in Tanzanian girls ages 9-14
- Measured in a VLP ELISA

K Baisley Lancet Glob Heath 2022

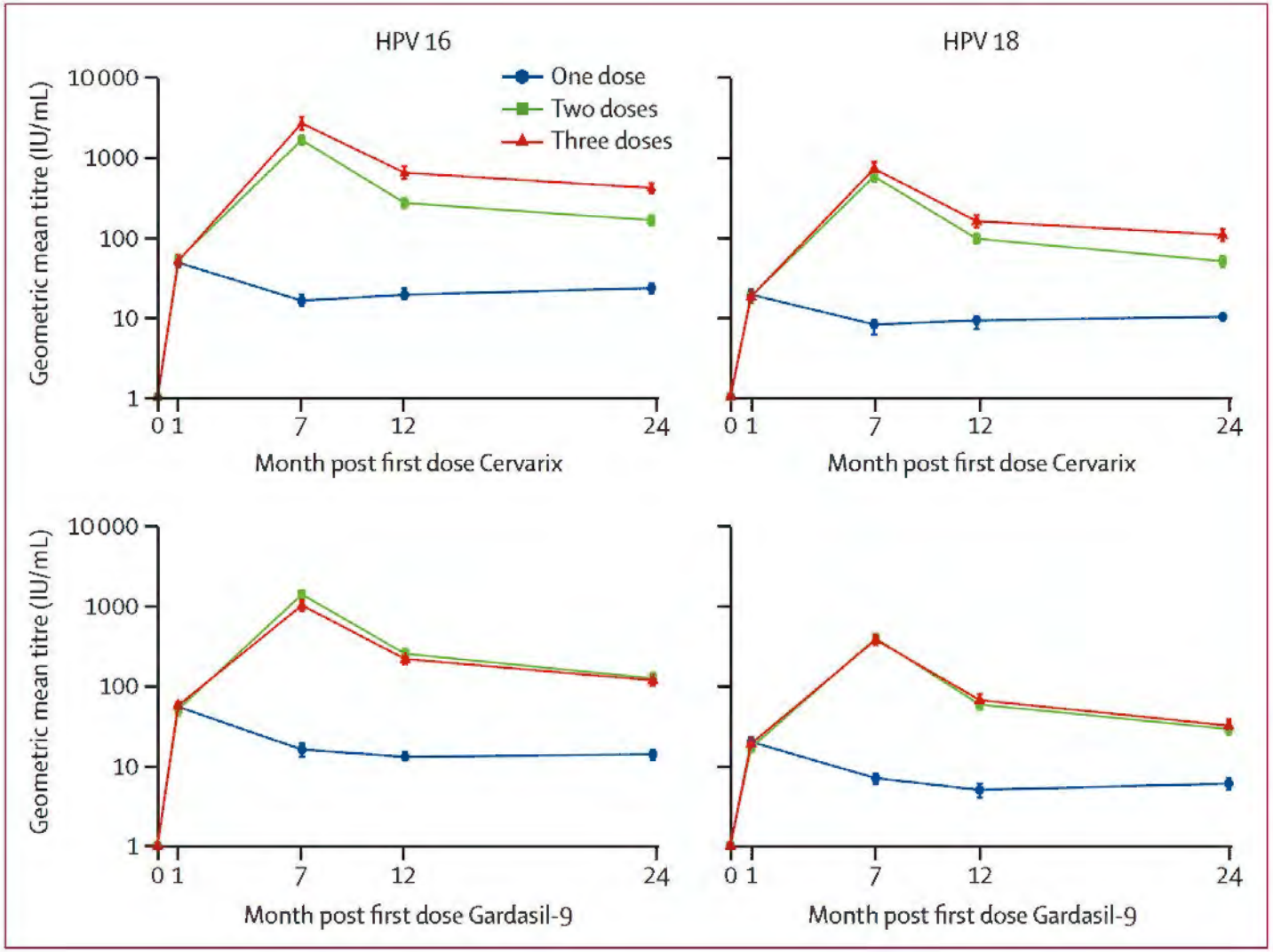
CVT: Stable HPV16 serum antibodies for 11 years

Results similar for HPV18



Kreimer AR et al, JNCI 2020

DORIS Trial: Ab Responses in Tanzanian Girls Ages 9-14



Cervarix

(Alum + MPL)

Gardasil-9

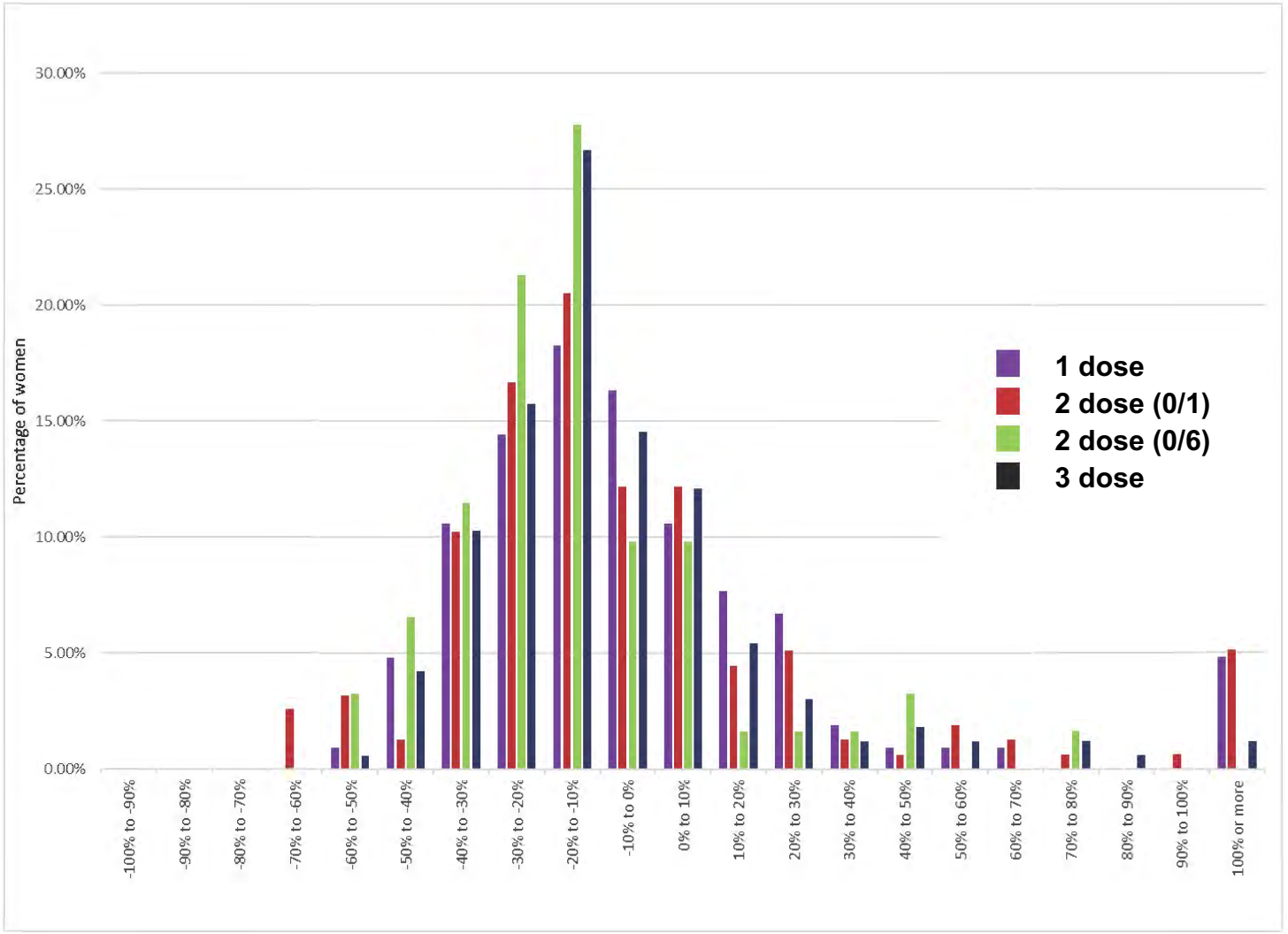
(Alum)

K Baisley Lancet Glob Heath 2022

Stabilization of Ab levels doesn't require a complex adjuvant.

Can environmental exposure to HPV virions account for the durable antibody responses?

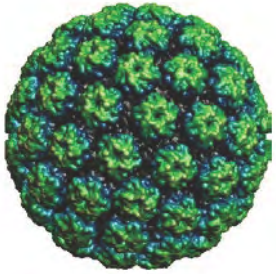
% Change in HPV16 VLP ELISA Titers From Year 4 to Year 7

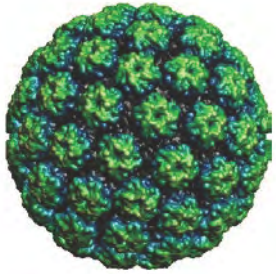


M Safaeian et al, J Nat Cancer Inst 110 (2), 2018

Why Are VLPs So Immunogenic?

They have Highly Repetitive Antigen Display





B cells specifically recognize particulate antigens with epitope spacing of 50-100Å as foreign.

This epitope spacing is commonly found on microbial surfaces, e.g. virus major capsid protein or bacterial pili.

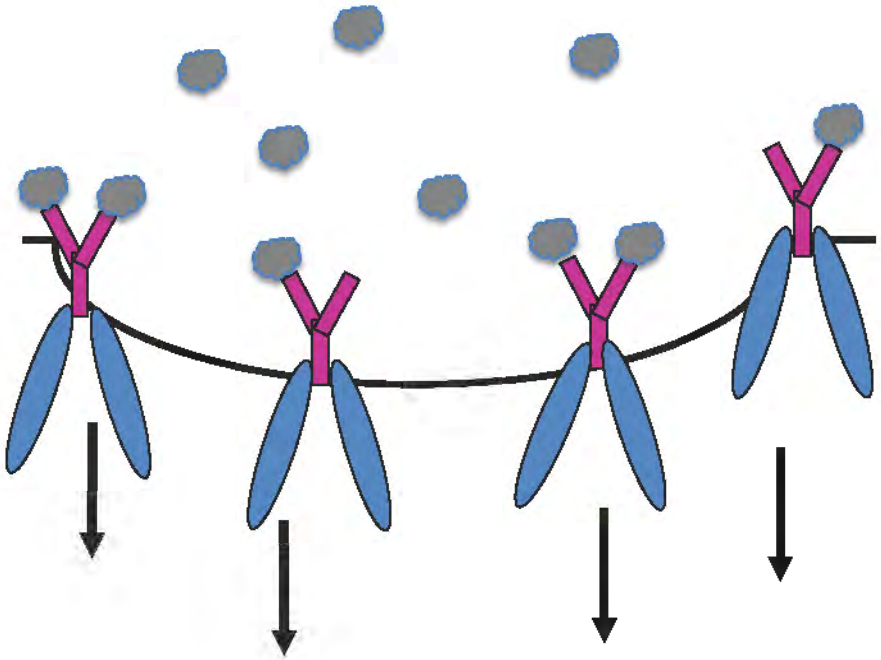
Protein complexes with this spacing rarely occur in vertebrate animals.

So BCRs have evolved as antigen specific pattern recognition receptors.

Bachmann et al. Science 1993; 262: 1448

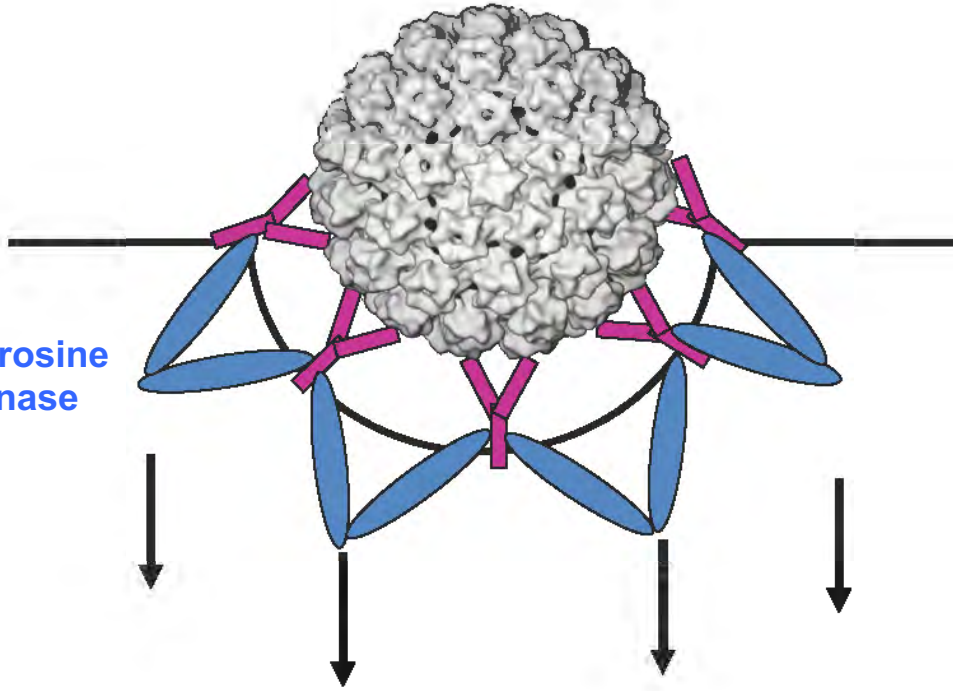
B Cells Recognize Dense Repetitive Protein Arrays as Dangerous Microbial Structures

Monomeric BCR/ Protein Complexes



Weak Activation Signals
Low Level Antibodies
Short duration

Oligomerization of BCR/Protein Signaling Complexes

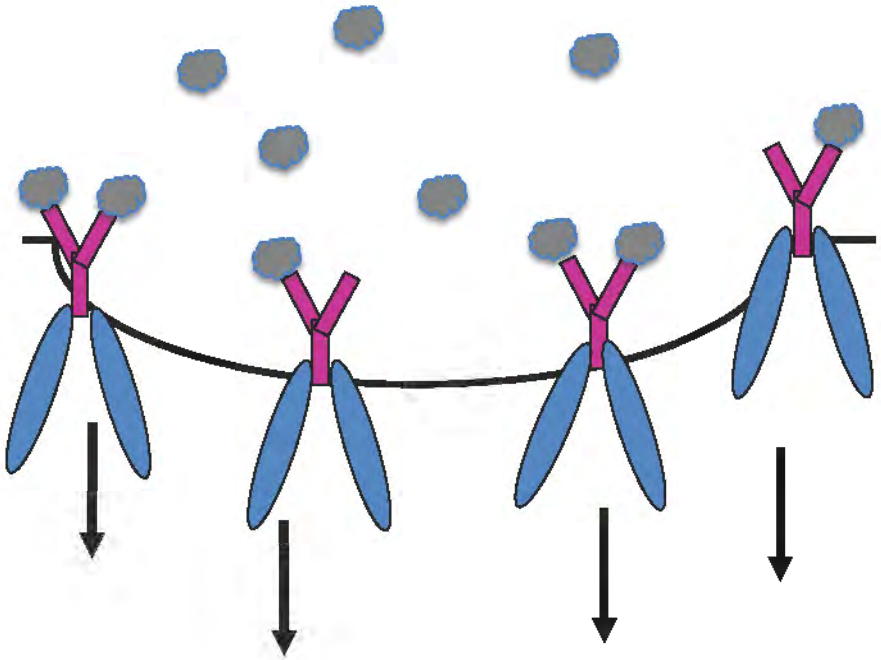


Strong Survival/Proliferation Signals
High Level Antibodies
Long Duration

Repetitive Ag structure guides the decision to invest in long term Ab production.

B Cells Recognize Dense Repetitive Protein Arrays as Dangerous Microbial Structures

Monomeric BCR/ Protein Complexes



Weak Activation Signals
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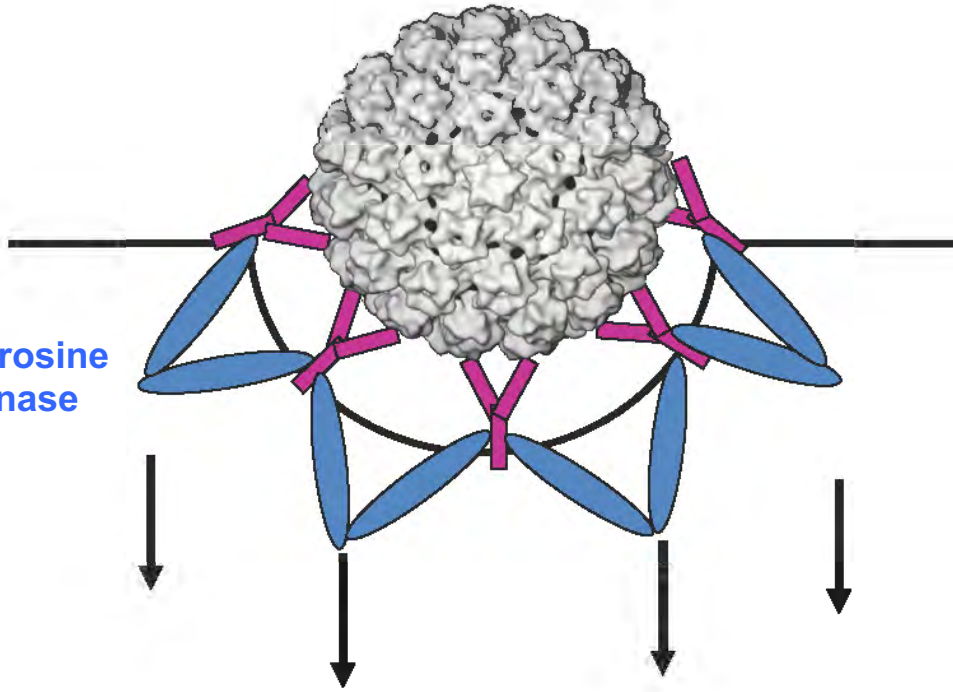
Oligomerization of BCR/Protein Signaling Complexes

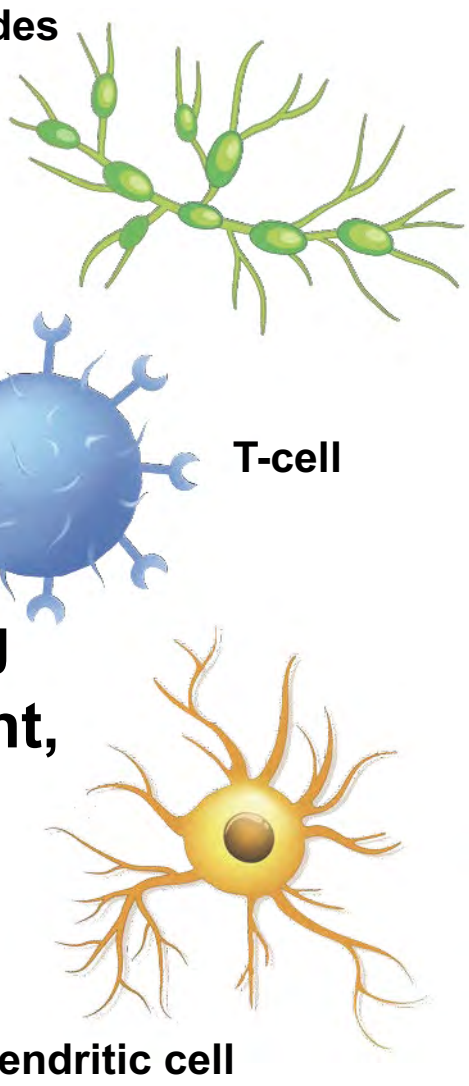


Strong Survival/Proliferation Signals
High Level Antibodies
Long Duration

What are the specific differences in signaling patterns?

Additional Advantages of VLPs

- They have the right particle size for efficient trafficking to lymph nodes
- They are readily phagocytized and so induce strong T-helper responses
- Their poly-valency leads to stable binding of natural low-avidity IgM and Complement, which promotes their acquisition by follicular dendritic cells*



*A. Link *J Immunol.* 2012; 188(8): 3724-33

Lessons For COVID-19 Vaccines?

Virus-like display of antigen, e.g. RBD, is the strategy most likely to consistently induce high titers of long-lasting antibodies.

ARTICLE

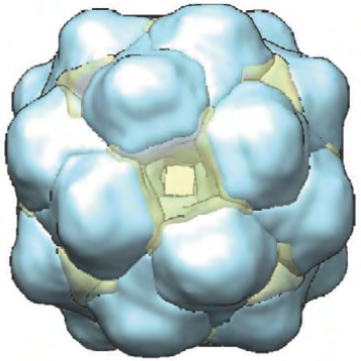
https://doi.org/10.1038/s41467-020-20251-8 OPEN

Capsid-like particles decorated with the SARS-CoV-2 receptor-binding domain elicit strong virus neutralization activity

Fougeroux et al. Nature Communication (2021) 12:324

The HBV VLPs Are Not As Immunogenic as HPV

- 1 dose often doesn't seroconvert.
- 3-dose titers wane over time.
- Good memory B cell response.

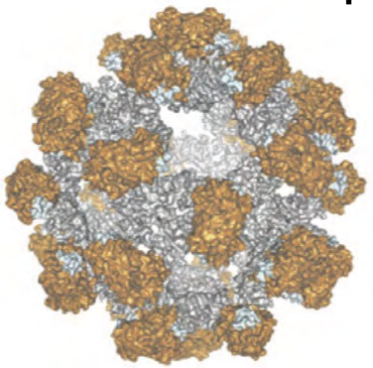


HBV S Ag

- 22 nM -

Same for Innovax's HEV VLPs

HEV p293



- 20 nM -

What's structurally "wrong" with these VLPs?

- Non-rigid lipo-protein structure?
- too few of repeats?
- too small, 22 nm?

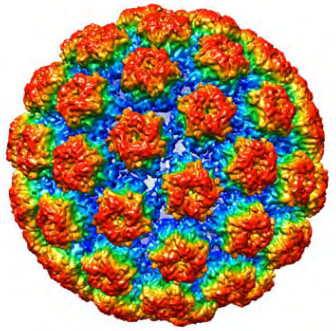
* Q Zhao, Trend Biotech 31:654-63, 2013

What structural features makes a polyvalent antigen “virus-like” to B cells?

More than closely spaced epitopes on a particle?

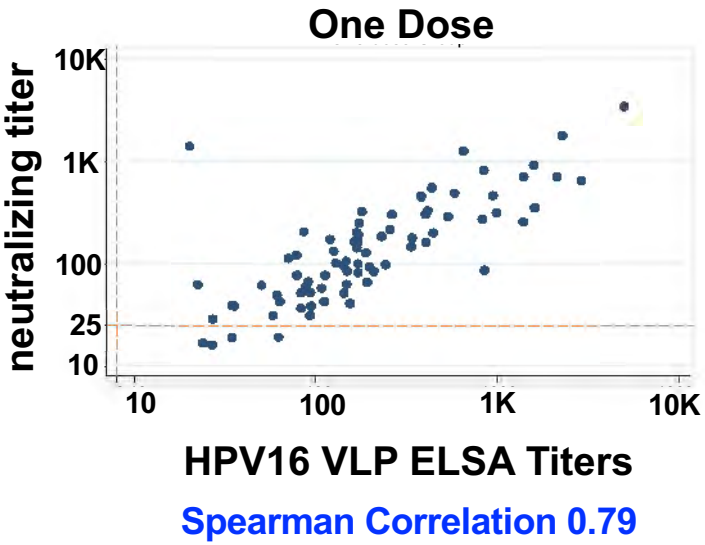
HPV VLPs:

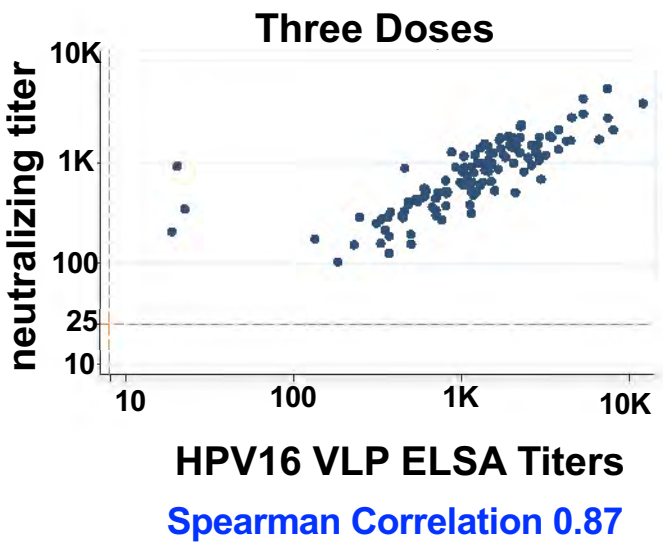
- **“Locked down” neutralizing epitopes**
- **Resistant to denaturation/proteolysis**
- **On a rigid platform**
- **How much flexibility in the platform or target antigen is tolerated?**



The Quality of the HPV VLP Abs Isn't Increased by Boosting

Correlation Between HPV16 ELISA and Neutralization Titers at 48 Months by Cervarix Dose

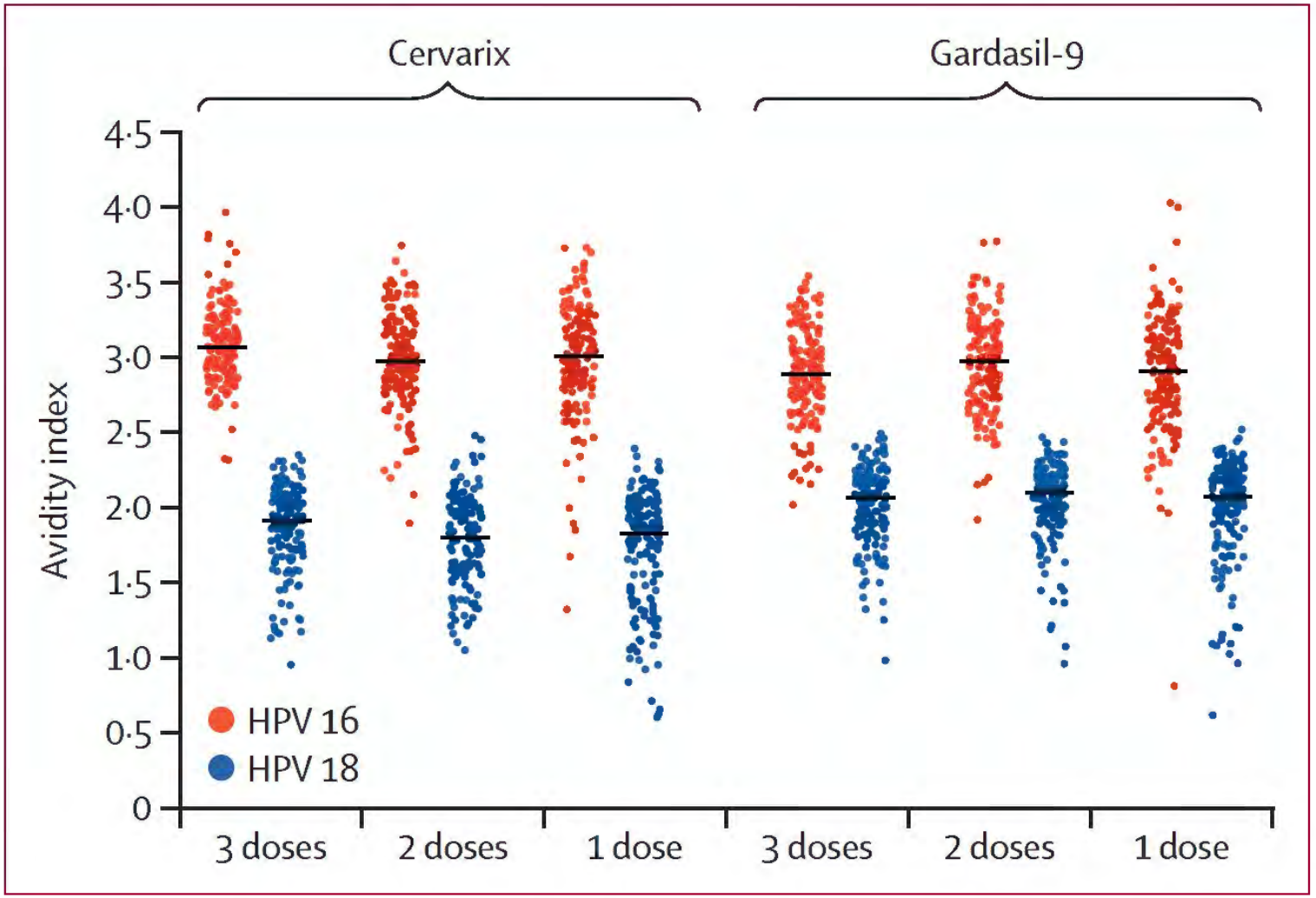




Ratios of Neutralizing to ELISA Titers similar, so high quality of the Ab response even after a single dose.

DORIS Trial: Boosts Don't Increase Ab Affinity

Guanidine HCl Chaotropic ELISA



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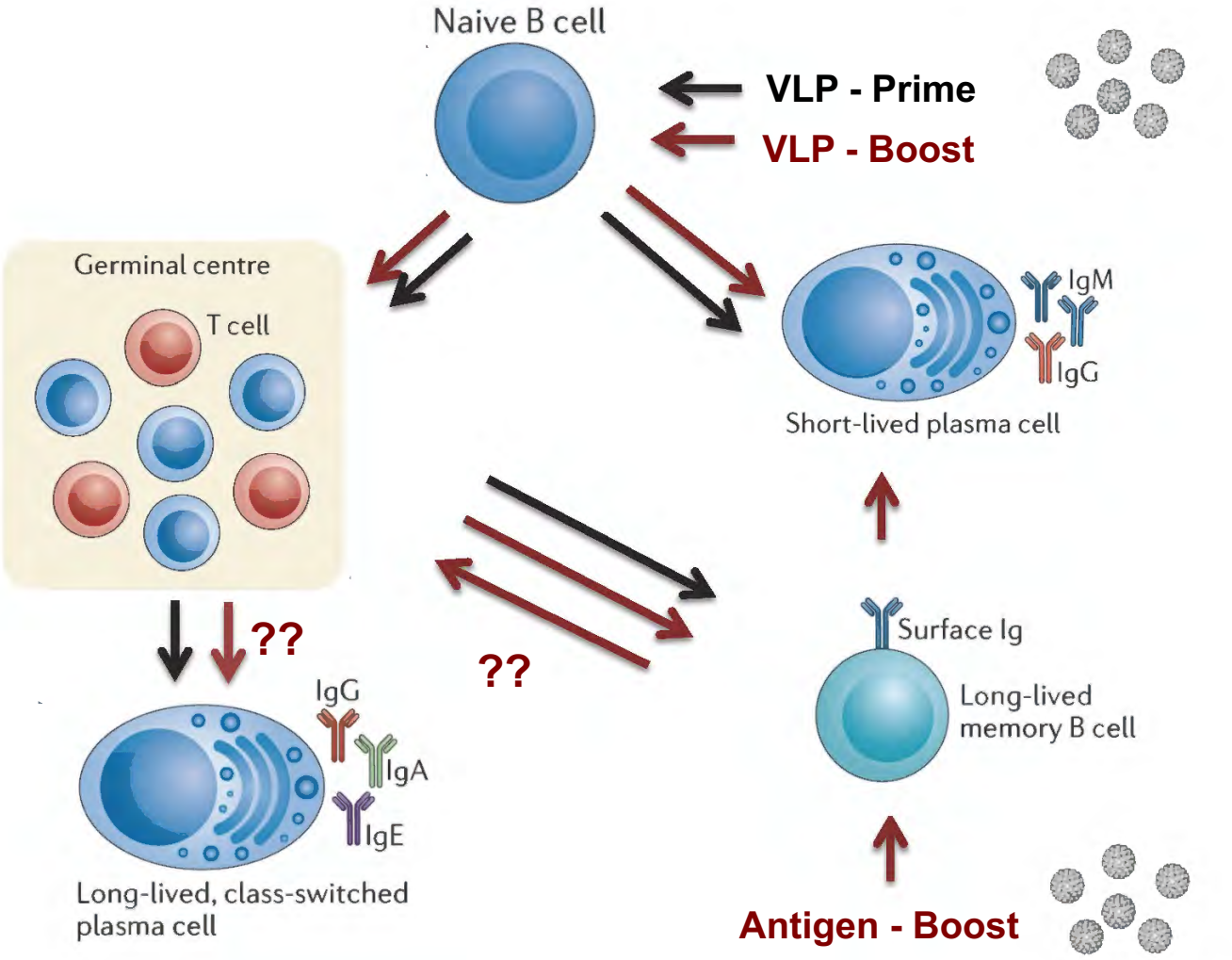
IF Boosting Doesn't Increase Ab Quality, Where Do LLPC Come From After Boosting?

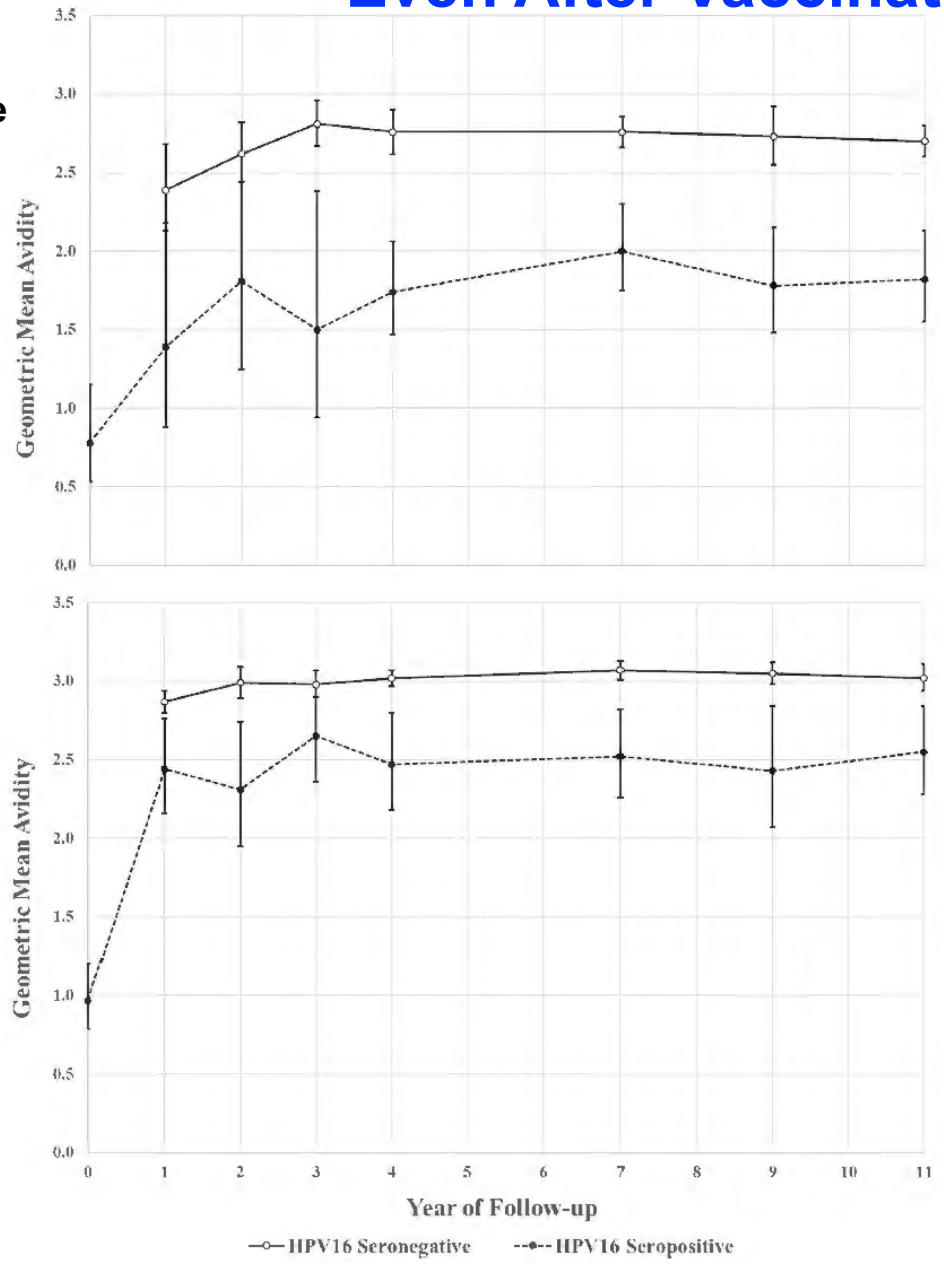


Hypothesis: VLP induced mBCs don't reenter GCs after reexposure to antigen, or if they do, they can't become long lived plasma cells.

Abs Induced by Infection Have Lower Avidity, Even After Vaccination

Single Dose

Three Dose



Results from Cervarix Vaccinees in CVT

Plateau titers don't vary by serostatus

Seronegative and positives are equally protected from new infections.

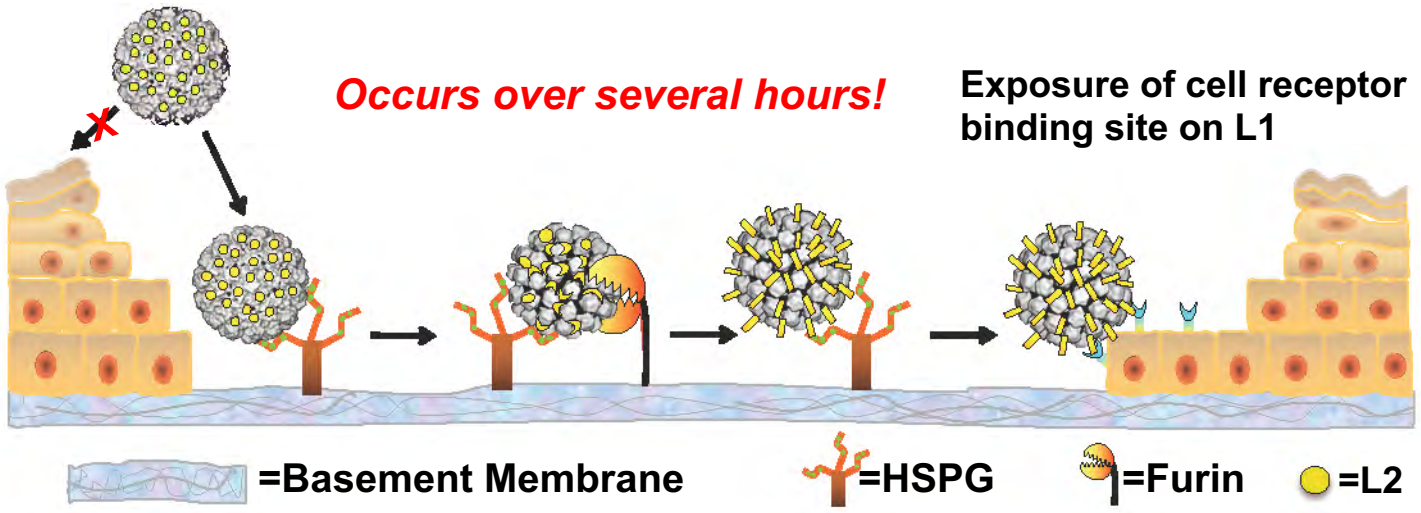
Do other asymptomatic mucosal infections reduce the overall quality of the Abs after vaccination?

Tsang SH NPJ Vaccines 2022

Virologic Aspects Contributing to Efficacy

In vivo Murine Model of Vaginal HPV Infection

The remarkably slow process of infection makes HPVs exceptionally susceptible to inhibition by antibodies



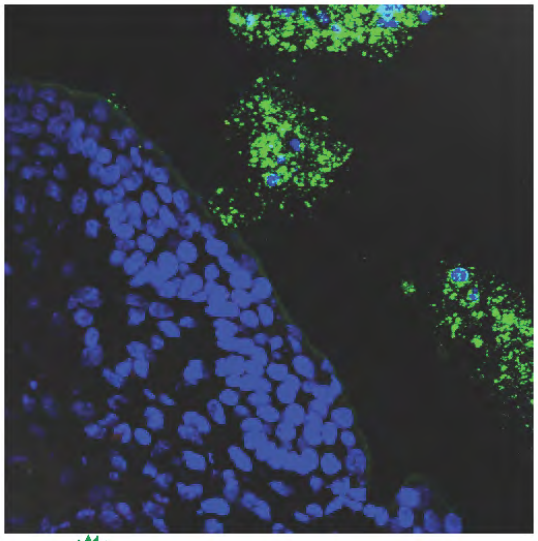
HSPG = Heparan Sulfate Proteoglycan

Rhonda Kines et al. PNAS 2009; 106:20458-63

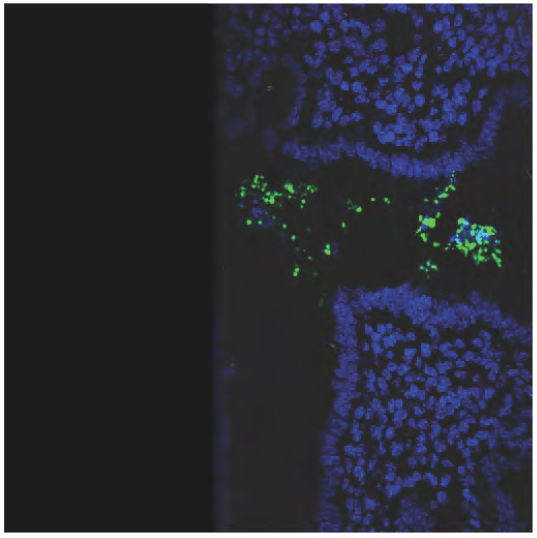
HPV Capsids Don't Bind Apical Surfaces of Intact Epithelium

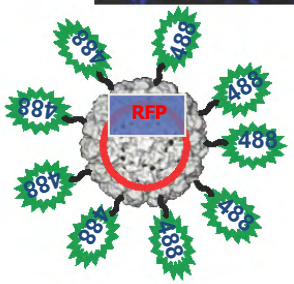
Mouse cervicovaginal challenge model

Vaginal Mucosa - stratified squamous



Endocervical Mucosa - simple columnar



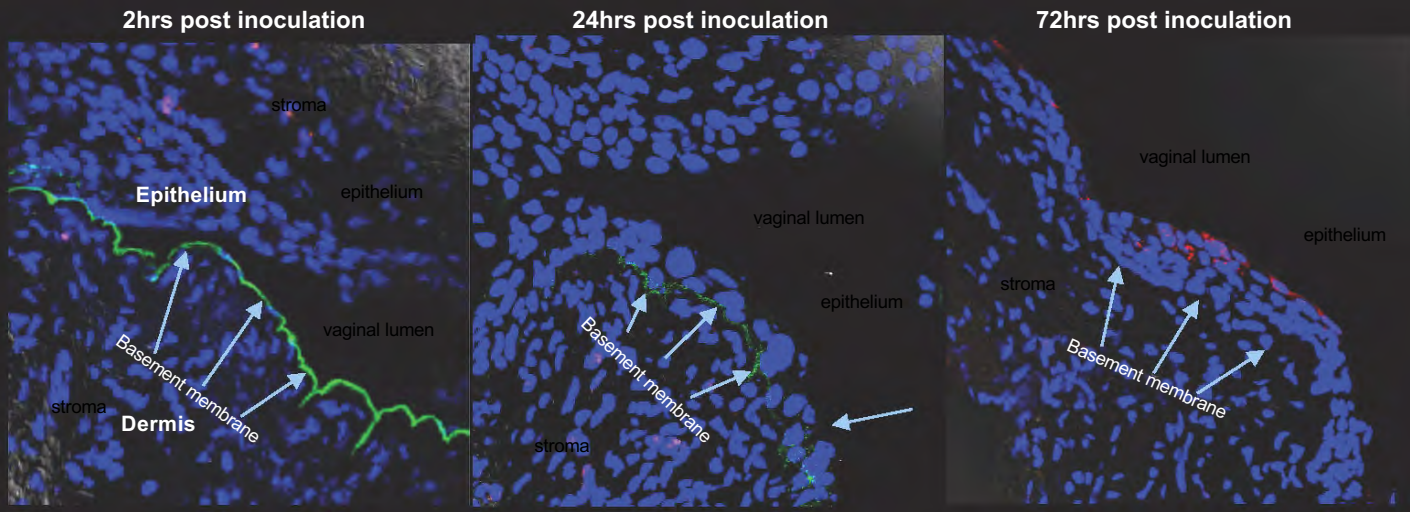


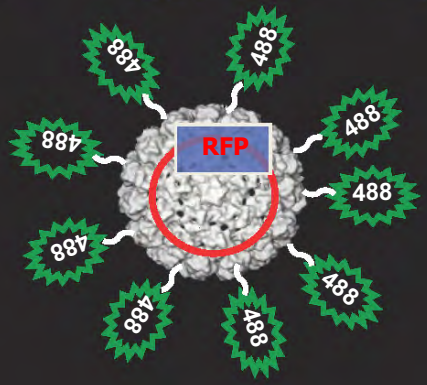
Green = (infectious) dye-coupled HPV capsids: binding

Red = RFP expression: infection

Roberts, et al. Nat Med. 2007 Jul;13(7):857-61

HPV16 Capsids Initially Bind Only to the Basement Membrane of Disrupted Stratified Squamous Epithelia in the Female Genital Tract





= PsV capsids

= RFP expression, infection

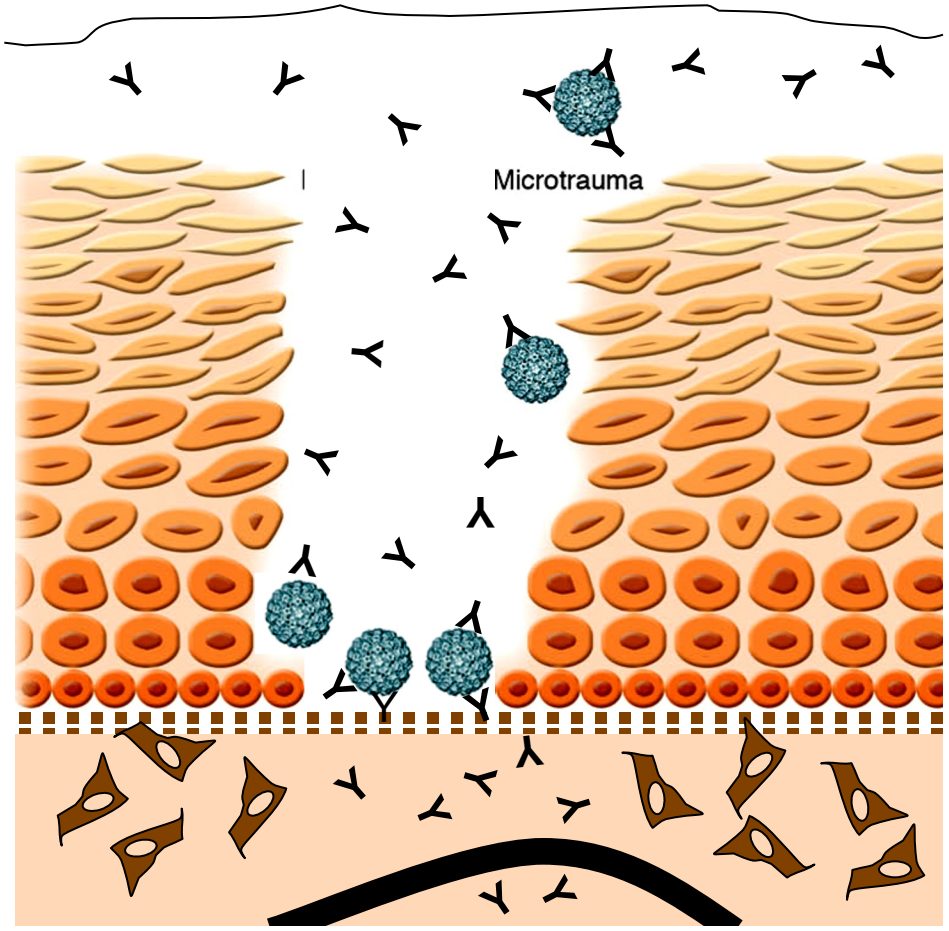
488 = alexa-fluor488 green fluorescent dye (PsV retains infectivity)

Roberts et al. Nat Med 13: 857-61, 2007

How Could IM Injection of a VLP Vaccine Induce a Protective Ab Response at the Cervix?

Transudated IgG Abs in Mucus (via FcRn)

Exudated Abs at Sites of Trauma



- VLP-specific IgG in women's cervical mucus after IM vaccination: but 10-100X less than in serum - *Nardelli et al. JNCI, 2003*
- Cervicovaginal HPV infection in a mouse model requires epithelial trauma: *Roberts et al., Nat Med, 2007*

Lessons For COVID-19 Vaccines?

Inducing sterilizing immunity will be much more difficult:

- **Covid directly infects the apical surface of the respiratory tract epithelium, so no Ab exudation.**

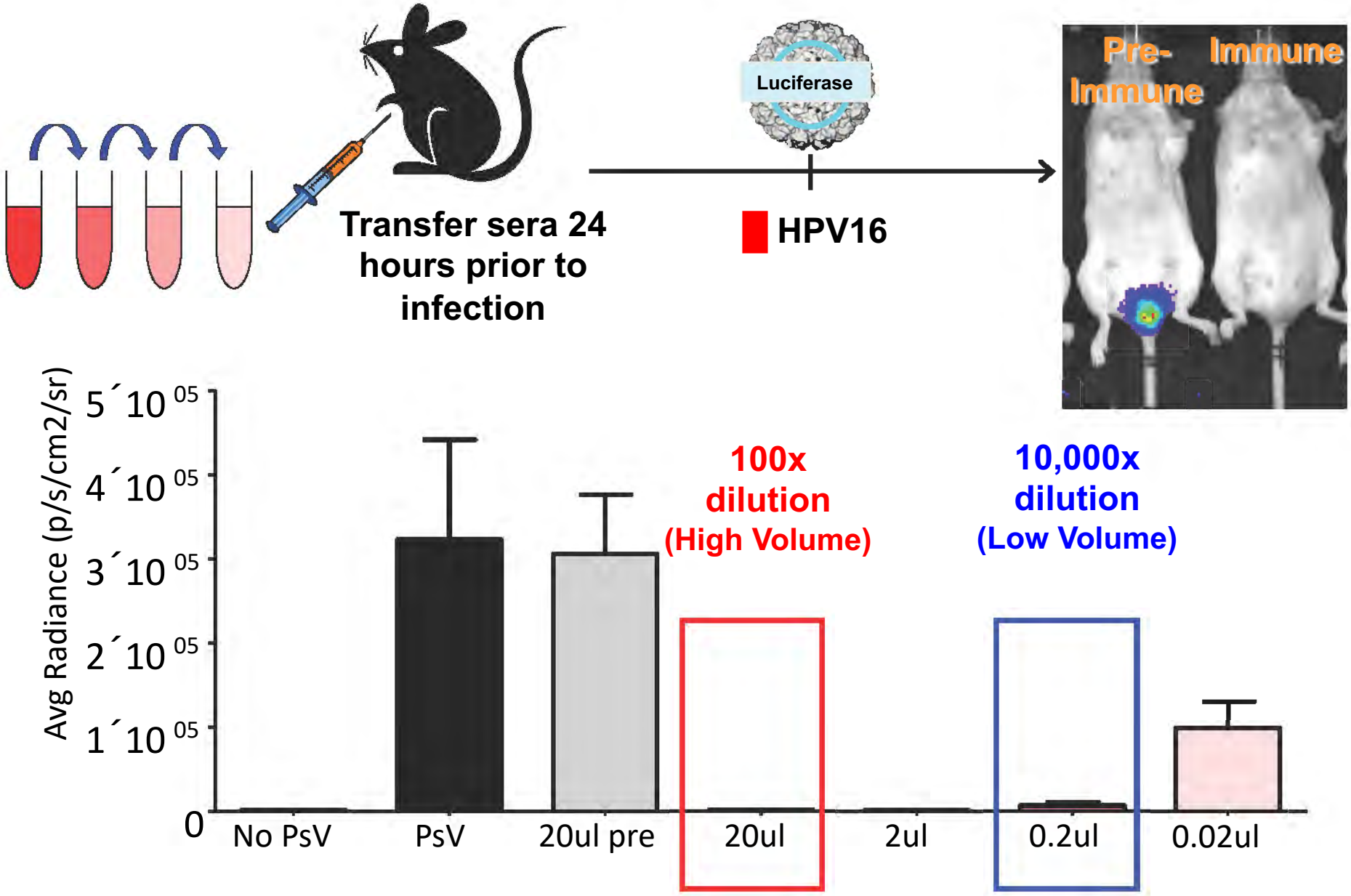
- **There is not extensive transudation of systemic IgG in the upper respiratory tract (although there is in the lung). Why the current vaccines protect better from serious disease than from initial infection?**

Antibody Titers and Protection

Are the plateau titers after vaccination near the minimum needed for protection?

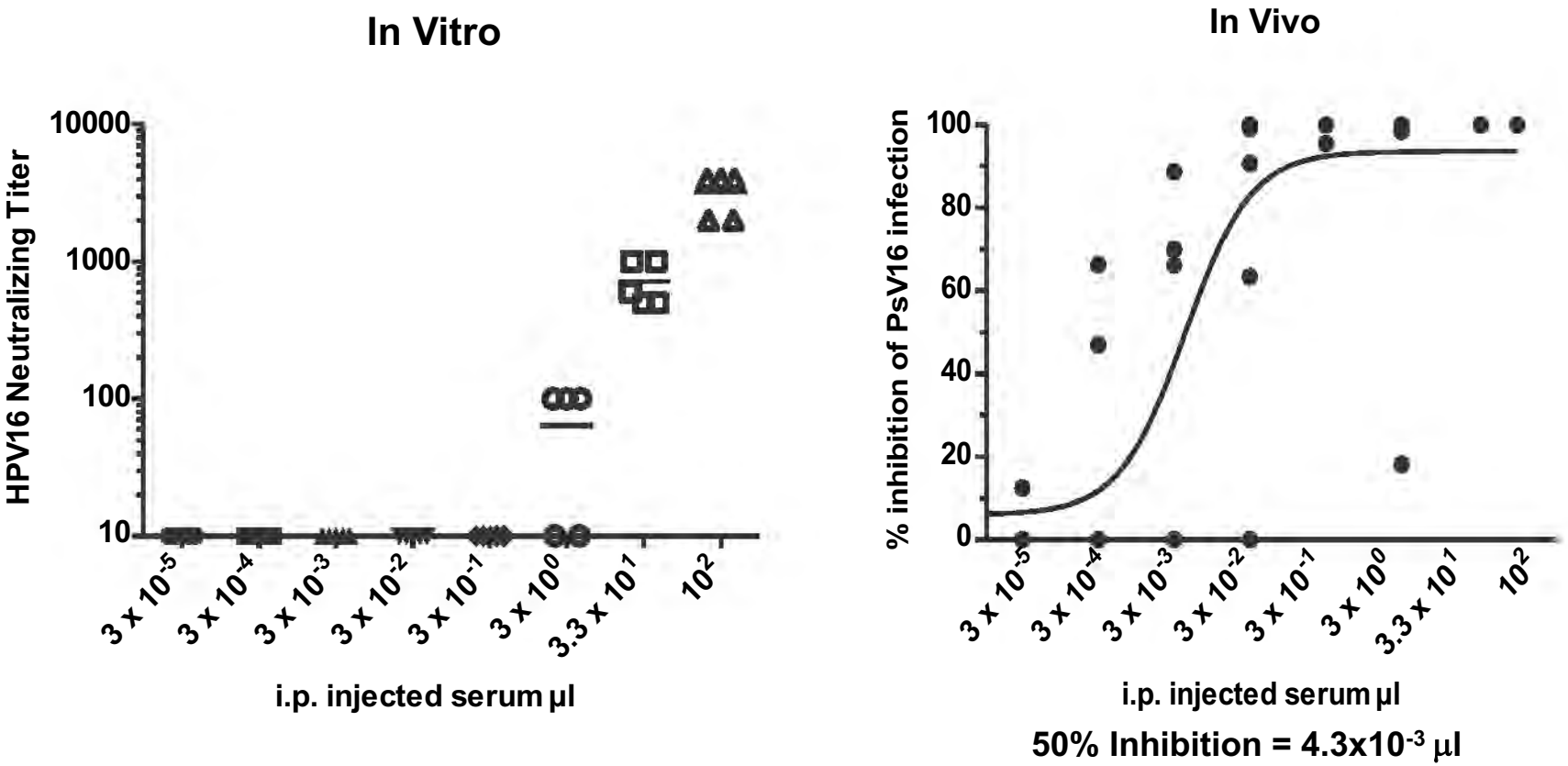
Will the 4-fold difference between Ab titers after three vs one dose influence long-term protection?

Passive Transfer of Rabbit Polyclonal Anti-16L1 VLP Sera



* Challenged with HPV16. See no protection from infection when challenged with HPV45

In vitro vs In Vivo Protection of Gardasil Sera Against HPV16 Pseudovirus Infection



Protection detected with ~100-fold less sera in vivo than in vitro!

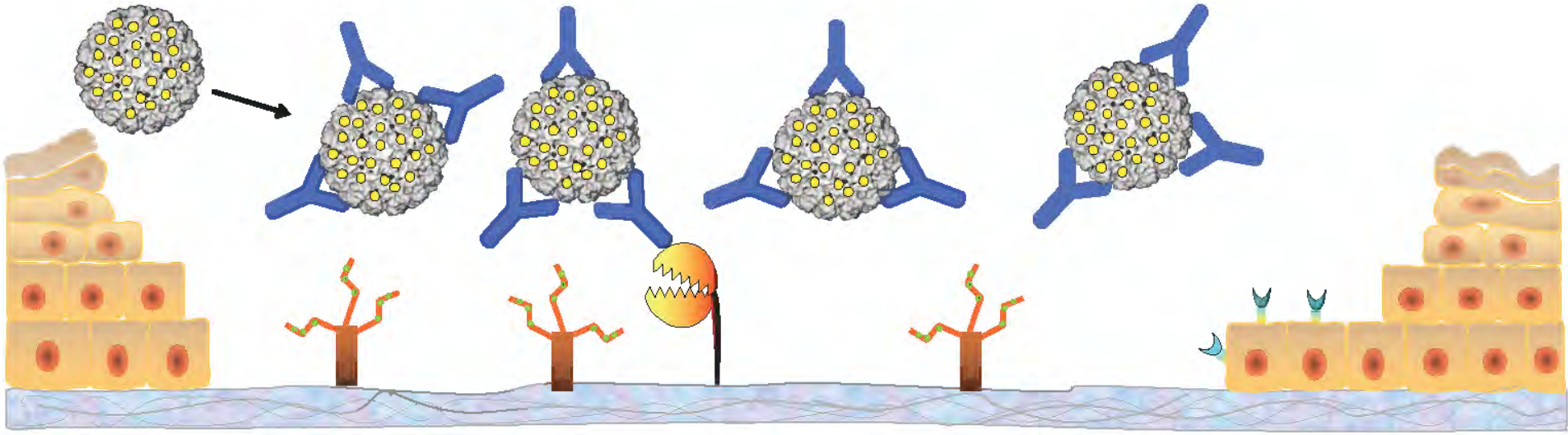
The in vitro assay is missing some potent mechanism of infection inhibition.

Longet et al, J Virol 2011

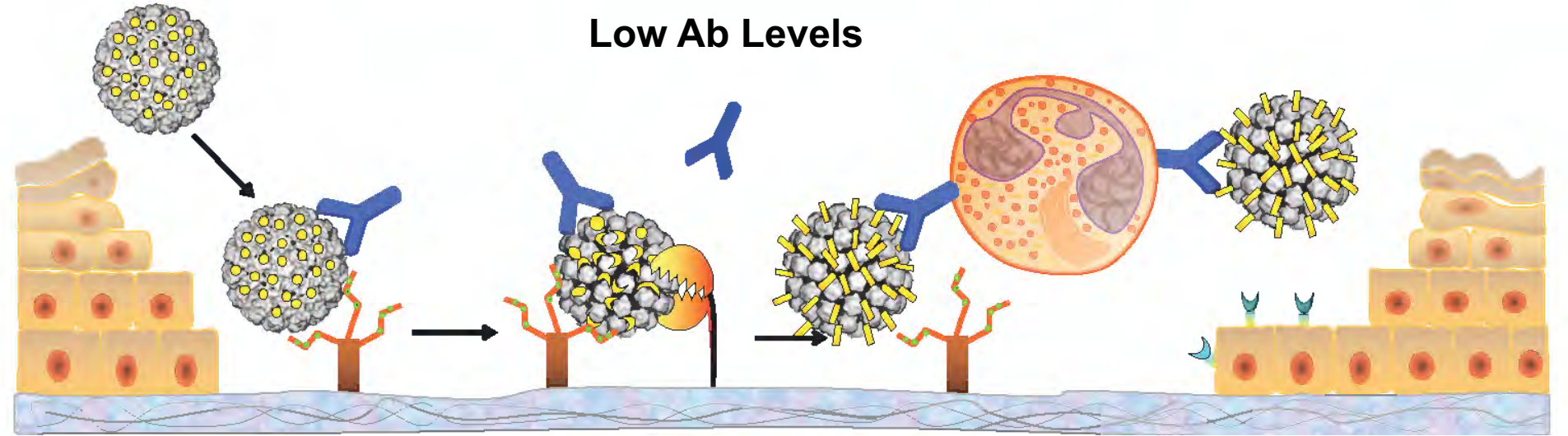
Mechanisms of In Vivo Infection Inhibition by VLP Abs

Day et al, Cell Host Microbe 2010; 8:260-70

High Ab Levels



Low Ab Levels



Conclusions

- The HPV VLP vaccines are very effective at preventing incident infection and disease by the vaccine types.

- Because:
  - the VLPs are exceptionally potent induces of neutralizing antibodies.
  - the virus is exceptionally susceptible to inhibition by antibodies.

- These observations provide important biological explanations as to why the HPV VLPs may become the first widely employed single-dose subunit vaccine.

- They provide a roadmap for developing vaccines against other infections that could similarly induce potent and long lasting antibody responses.

Key Collaborators

Present Members of the Lab:

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