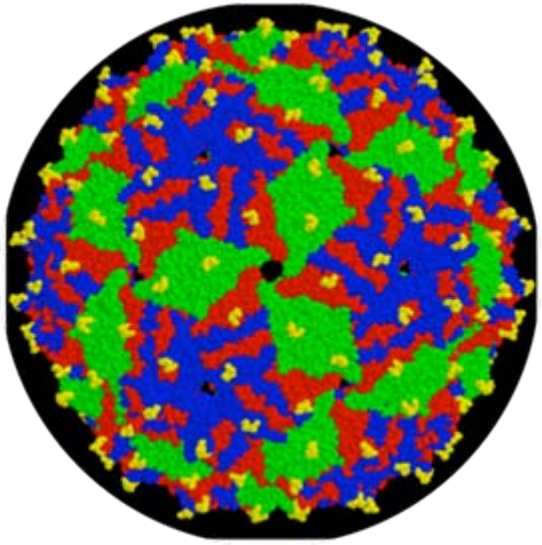
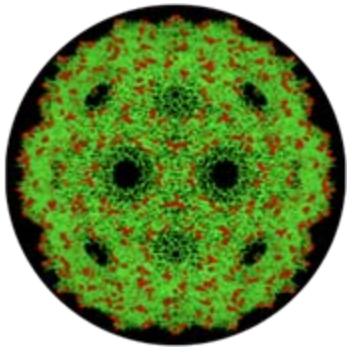
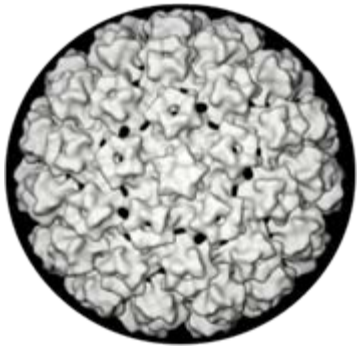






VLPs are highly immunogenic

- Particulate antigens with highly dense, repetitive spacing are commonly found on microbial surfaces (such as virus particles or bacterial structural proteins), but rarely occur among self-proteins.

- Hypothesis:
  The humoral immune system responds vigorously to dense repetitive arrays, leading to B cell proliferation and the production of antibodies.

- Focus of today: Using virus particles as a platform to increase the immunogenicity of target molecules that are poorly immunogenic.

VLPs as Platforms for Multivalent Display of Target

Potential Targets:

- Conventional targets
  - Peptide epitopes or domains derived from pathogens
- Non-protein targets
  - Such as carbohydrates or chemical agents
- Self-Antigens
  - Self-molecules involved in chronic and infectious diseases

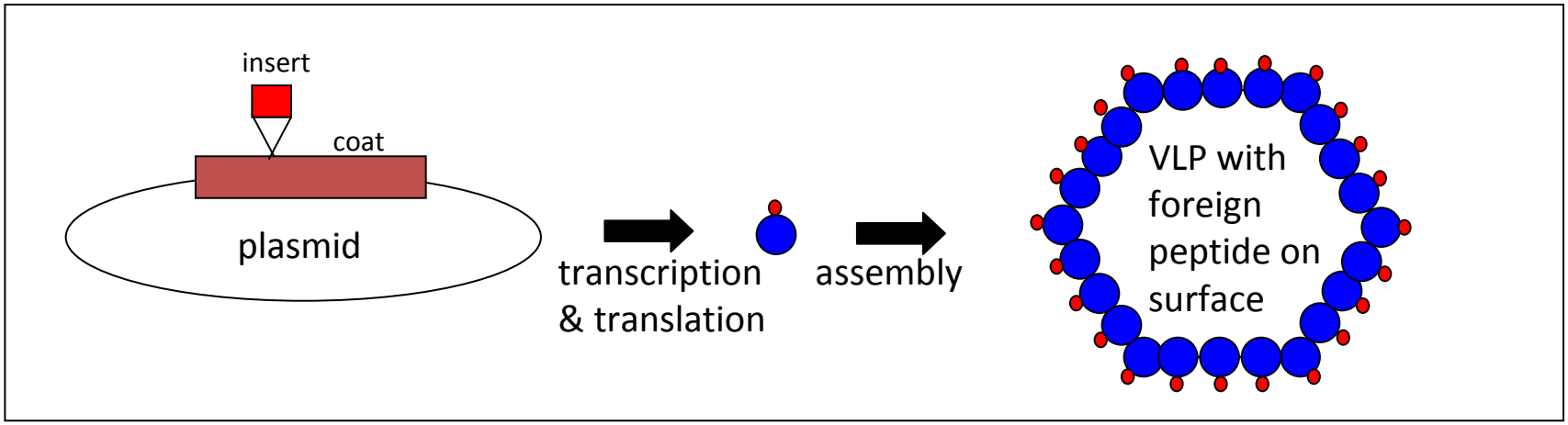
Classes of antigen:

- Short peptides (or single epitopes)
  - Antibody responses essentially mimic monoclonal antibodies
  - Simpler to display on VLPs
  - Structural considerations

- Large protein domains
  - Elicit polyclonal responses
  - Better mimic of native structure
  - More difficult to display on VLPs

How do we display heterologous antigens on VLPs?

1) Genetic insertion of target peptides into viral structural proteins

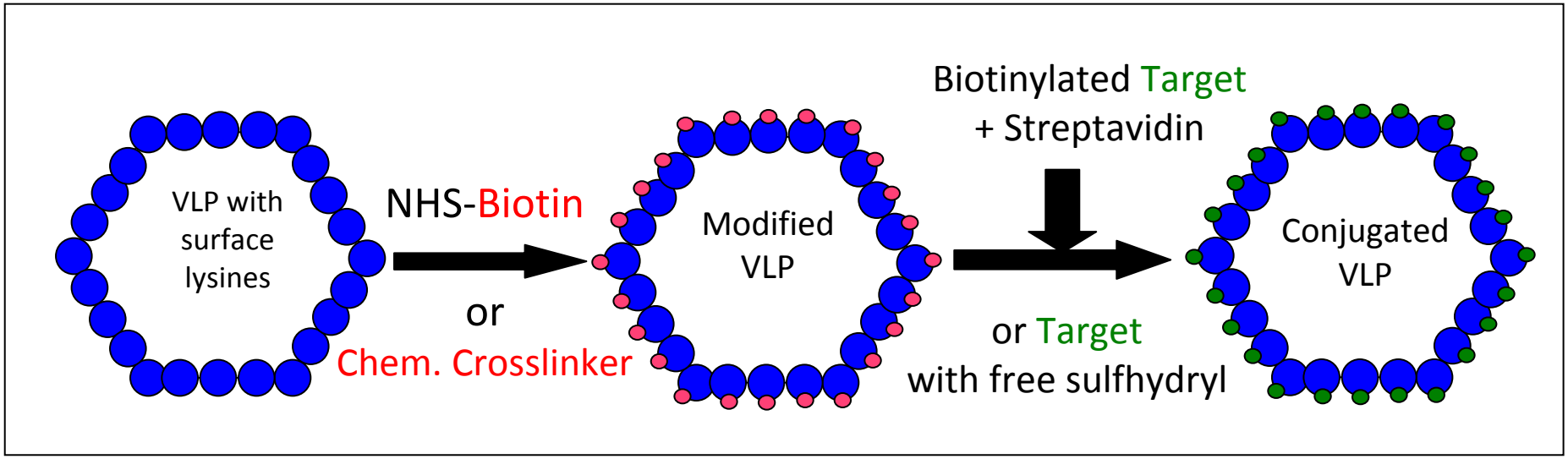


Advantages: Guarantees regular display
Manufacturing advantages

Disadvantages: Insertions often incompatible with VLP assembly
Size of insertion limited (usually restricted to linear epitopes and mimotopes)
Limited to peptide insertions

How do we display heterologous antigens on VLPs?

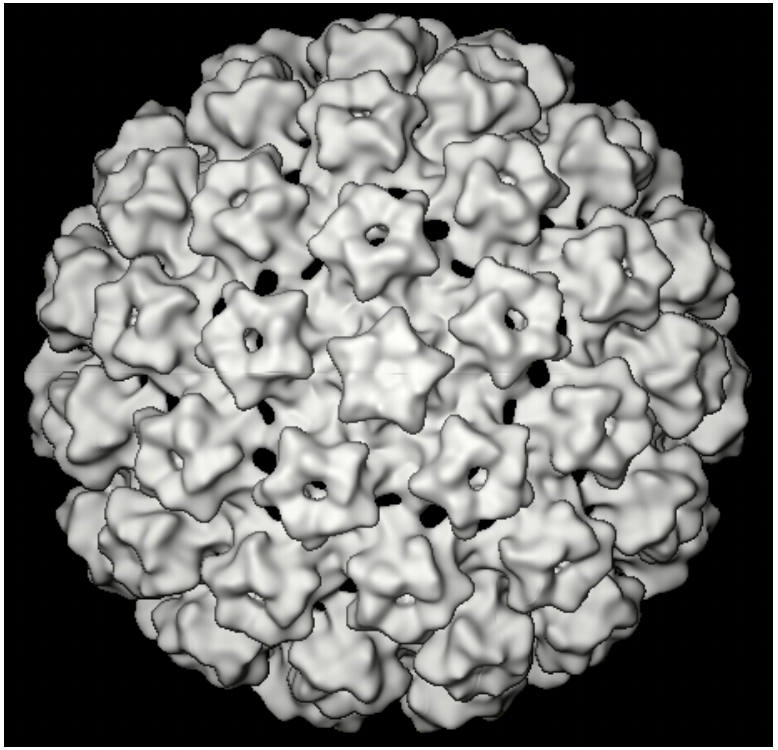
2) Chemical Conjugation



Advantages:
Can take advantage of a variety of linking chemistries
Can target diverse sizes and types of antigens

Disadvantage:
More complex manufacturing
Surface chemistry of VLP may preclude use of chemical crosslinkers.

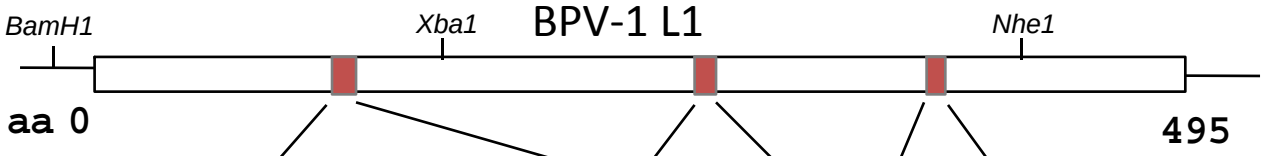
VLP Platform Technologies: Papillomavirus



- T=7 Particles, consisting of 72 pentamers of the major capsid protein, L1.
- Can be produced using mammalian, insect, and yeast expression systems.
- The basis for the current HPV vaccines Gardasil and Cervavix.
- Can be derived from diverse human and animal papillomavirus types.

Today: (1) PV Display Technologies, (2) Immunogenicity of modified PV VLPs

Chimeric Papillomavirus VLPs: 16 aa CCR5 peptide



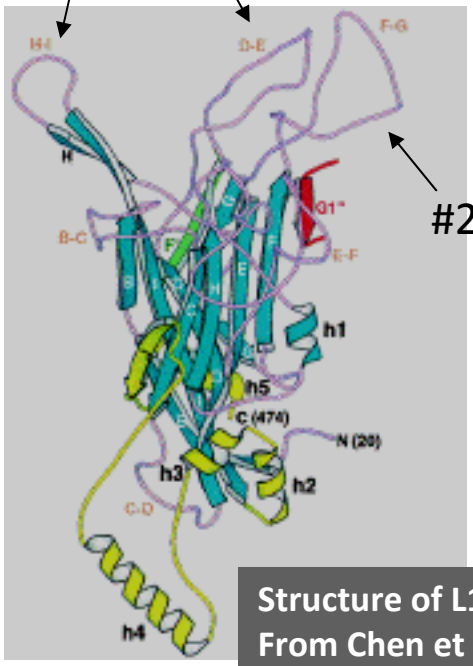
aa**126** ENVN---**RKVTTQT**-----TDDRKQ **142**
#1 ...**HYAANEWVFGNIMCKV**.....

aa**271** FYLK--**NNKGDATLKIP**---SVH **288**
#2 ...**HYAANEWVFGNIMCKV**...

aa**340** TISV---**ASDGTPL**-----TEYDS **355**
#3 ...**HYAANEWVFGNIMCKV**.....

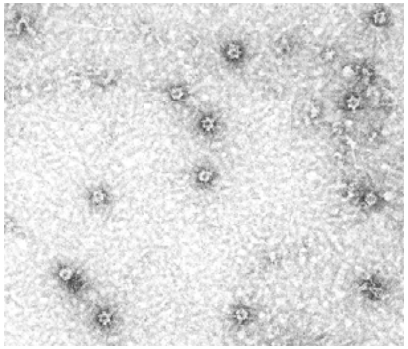
Construct	Particles?
#1	Yes
#2	No
#3	No

#3 #1 #2

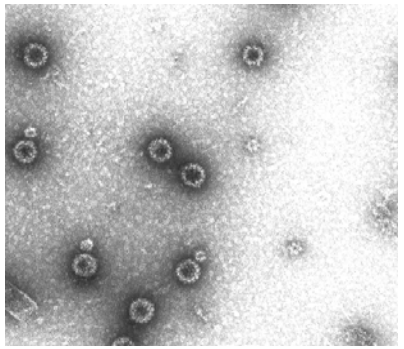


Structure of L1 Monomer
From Chen et al., Molecular Cell 2000

Construct #1



BPV-1 VLPs



Chackerian et al., PNAS 1999

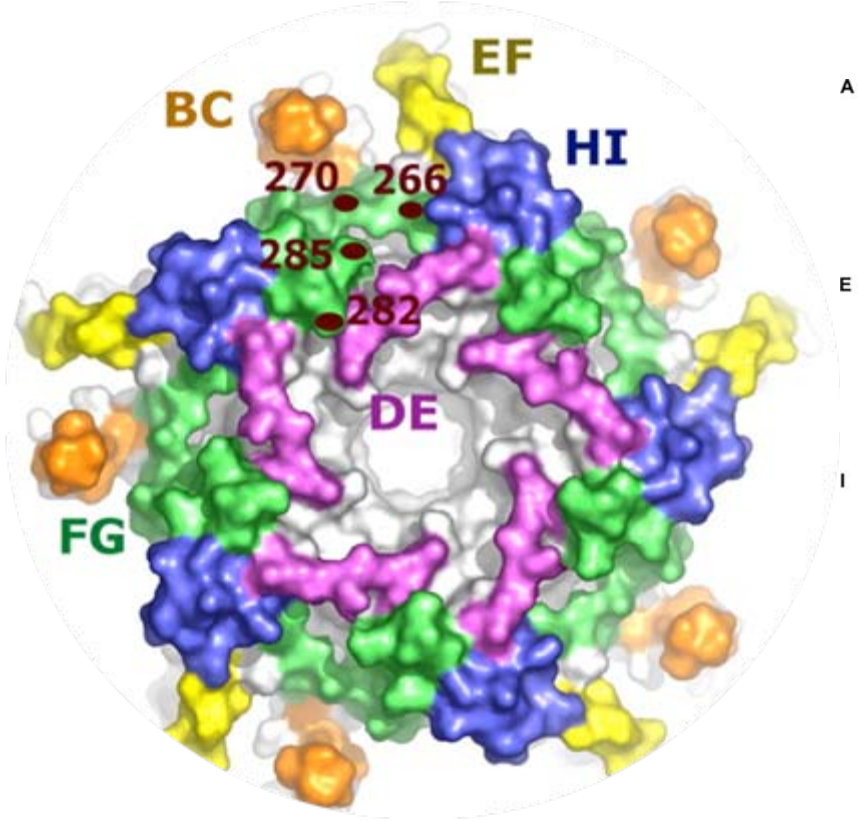
Immunogenicity of recombinant PV VLPs

Immunogen	Adjuvant	Anti-CCR5 End-point dilution Titer
L1-CCR5	CFA	3×10^4
L1-CCR5	None	3×10^3
Denatured L1-CCR5	CFA	<40
w.t. BPV L1 VLPs	CFA	<40

Groups of mice were immunized 3 times with 10 μg of VLPs 3 times at two-week intervals.

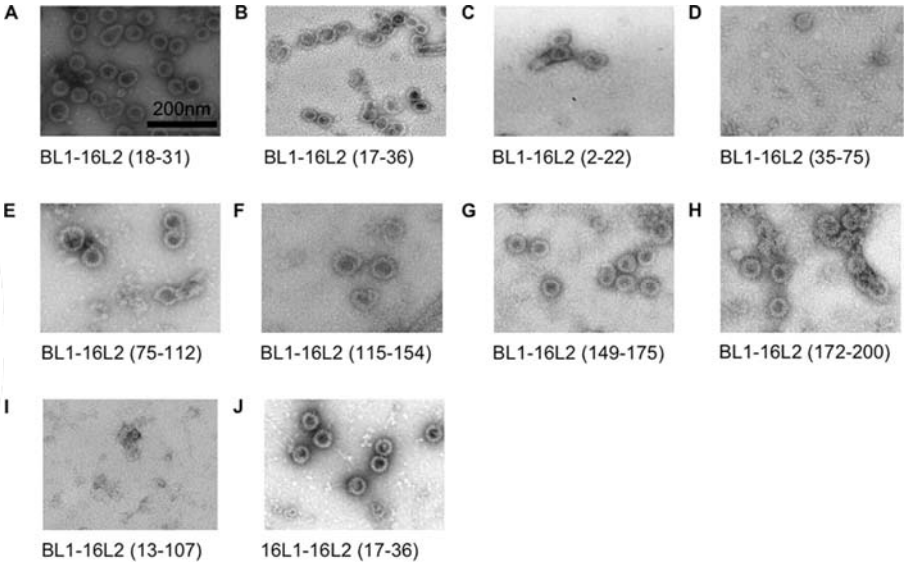
Chimeric Papillomavirus VLPs

HPV 16 pentamer



Bishop et al., JBC 2007

BPV-1 Chimeric VLPs
(inserted into DE-loop)



Anti-L2 Antibody Titers:

A: 62,500	E: 2,500
B: 62,500	F: 62,500
C: 0	G: 62,500
D: not tested	H: 312,500
	I: 500

Schellenbacher et al., J Virol 2009

Recombinant PV VLPs: Summary

- Chimeric VLPs based on BPV-1 can be constructed by inserting target peptides into the exposed DE-loop.
- Peptides as large as 40 amino acids can be inserted into this site.
- Lack of systematic testing of ability to generate chimeric PV VLPs
- Chimeric VLPs can induce high titer antibody responses against self- and non-self targets, even in the absence of adjuvant.

Chemical Conjugation to Papillomavirus VLPs: Targeting self- and foreign antigens in the HEL Tg Mouse Model

(Chackerian et al., J Immunol 2008)

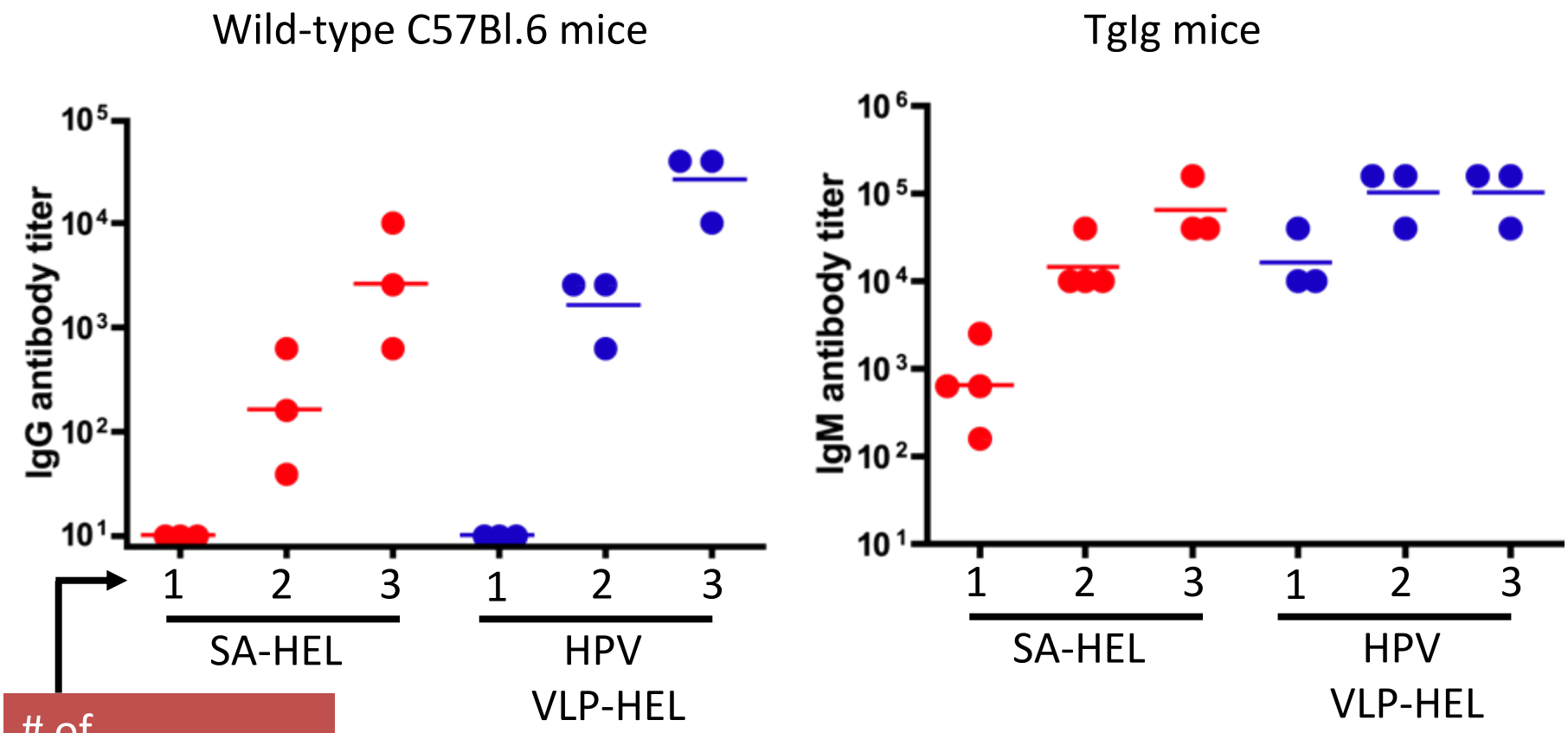
Immunogen:

- Biotinylated HPV VLPs linked at high valency to biotinylated HEL using the streptavidin "bridge" technique. Resulted in ~0.5-1 copies of HEL per L1 molecule.
- As a low valency control, HEL linked to streptavidin alone (low valency).

Mice:

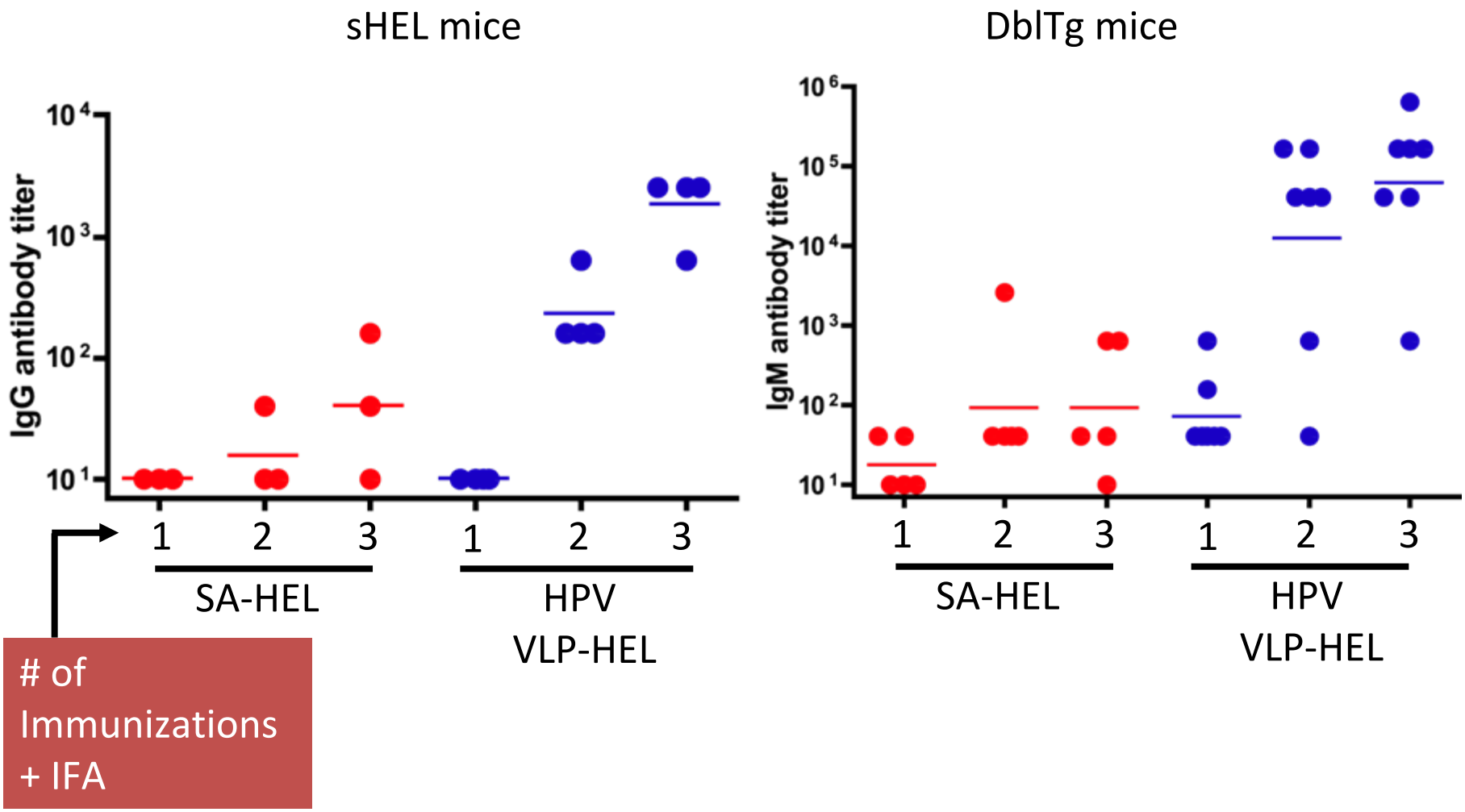
Mouse Strain	Description	B cells
sHEL	Express soluble HEL as a neo-self-antigen	Anergic, Normal repertoire
TgIg	Transgenic for B cells that are specific for HEL	Responsive (not anergic) Monoclonal
Dbl Tg	sHEL x TgIg	Anergic, Monoclonal

Responses in non-tolerant mice

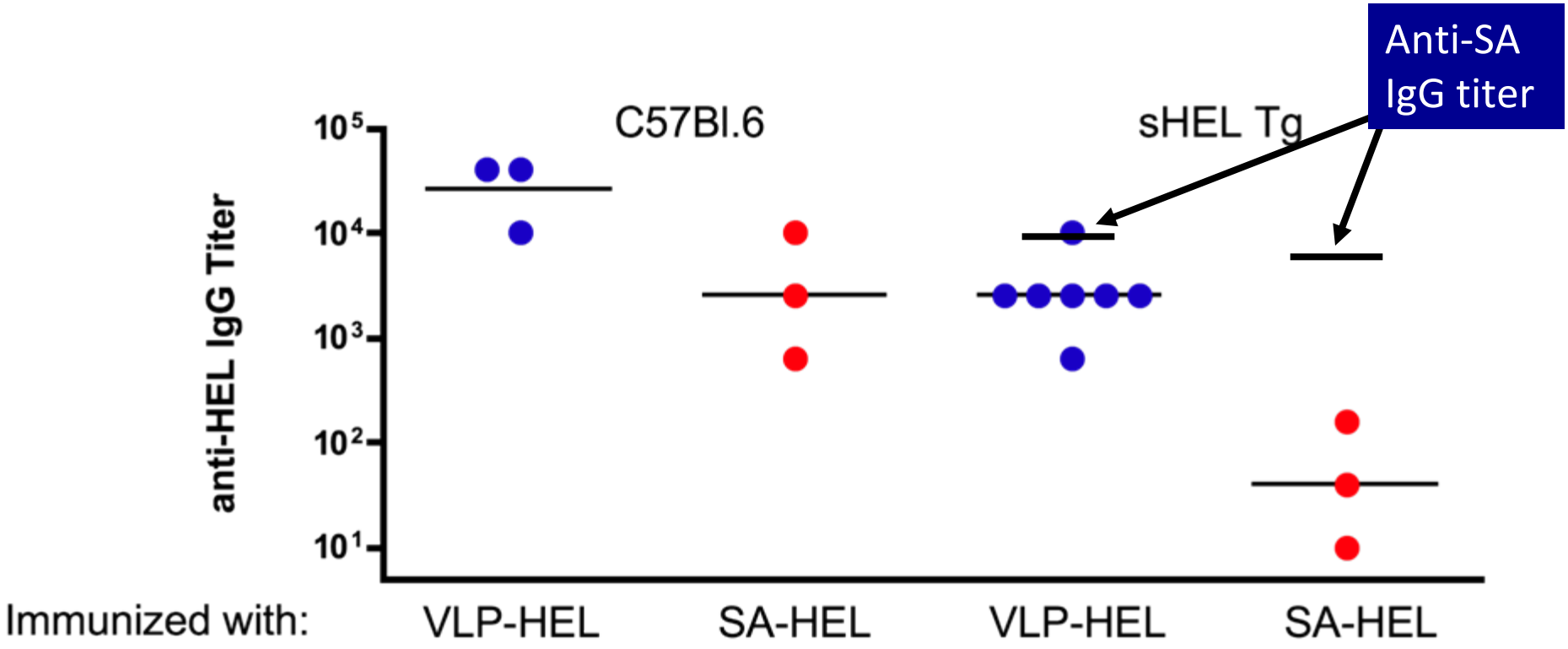


Sera taken 1 week after each immunization

Responses in Tolerant mice

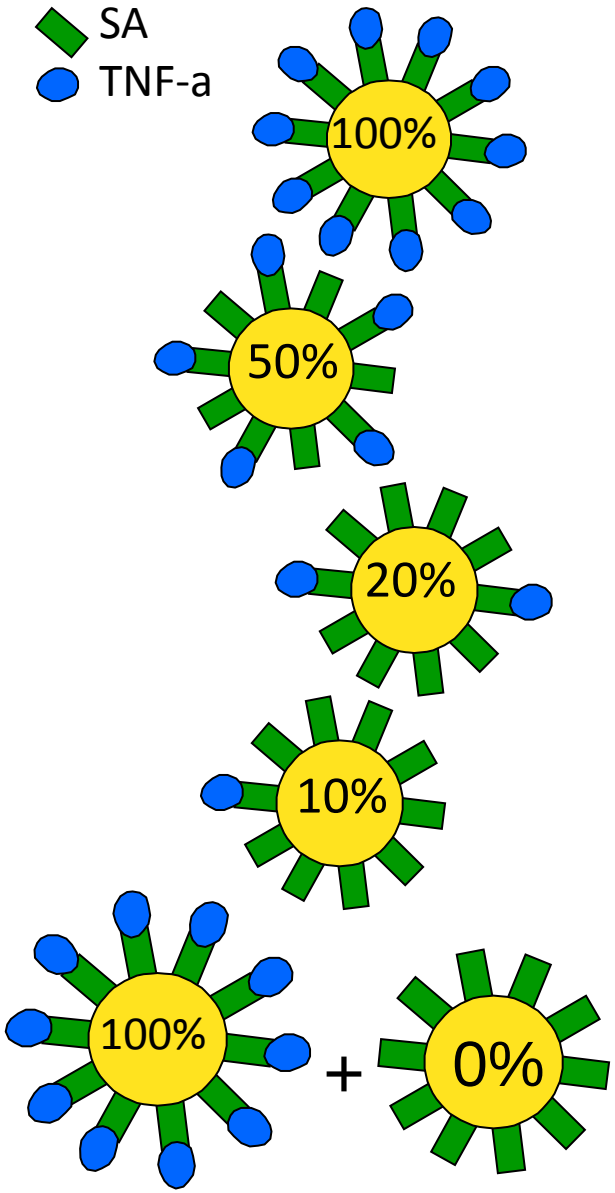


Comparison of responses in Tolerant and non-tolerant mice



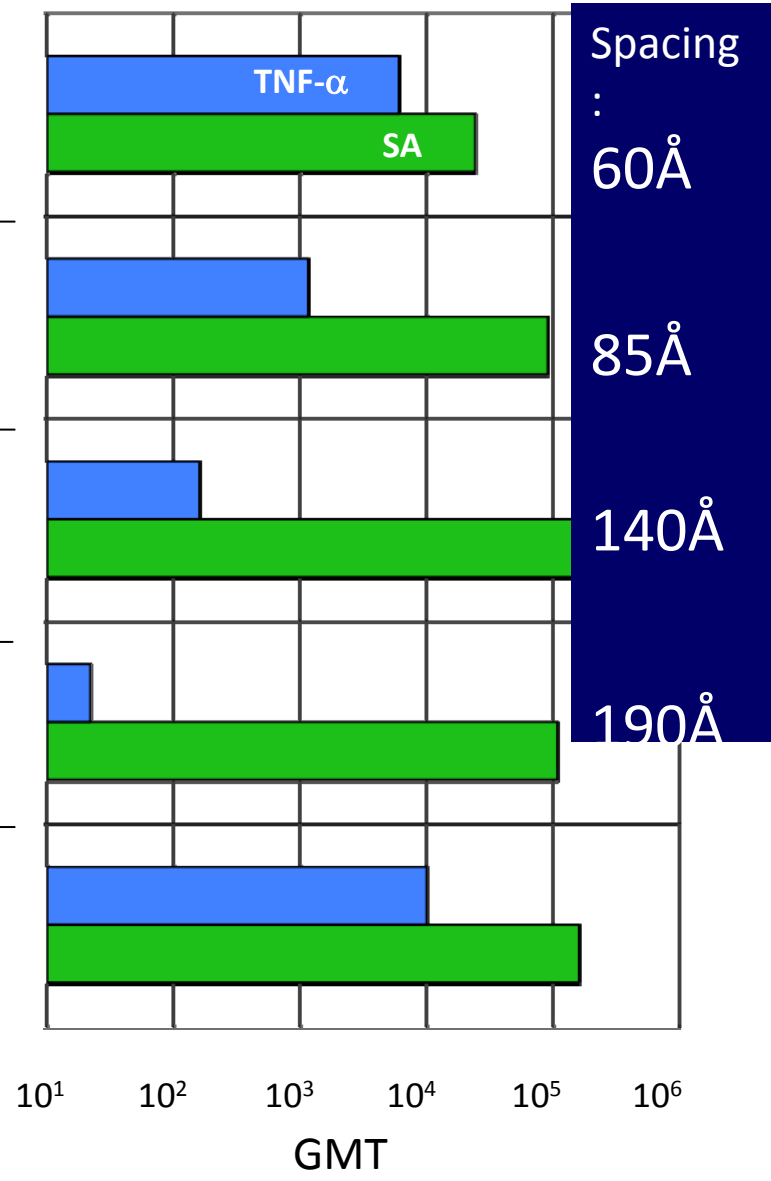
Similar results targeting CCR5, TNF-α, Amyloid-beta, gastrin and others

The Density of Self-antigen on VLP Surfaces Effects Autoantibody Responses



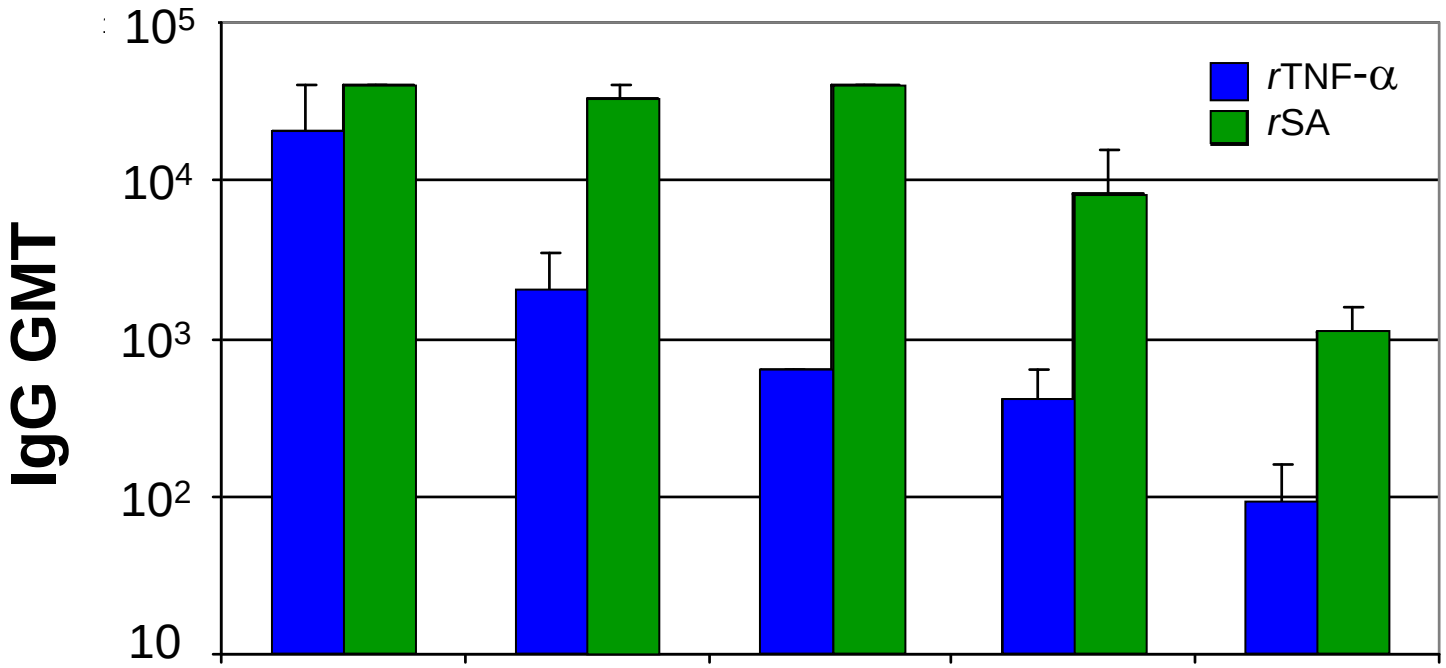
µg per injection

VLP	SA-TNF-α	SA
1	2	0
2	2	2
6	2	8
10	2	18
1	2	0
9	+ 0	18



Chackerian et al., J Immunol 2002

Differential Antibody Responses Against Self- and Foreign Antigens Using Particles Conjugated at Sub-Maximum Densities

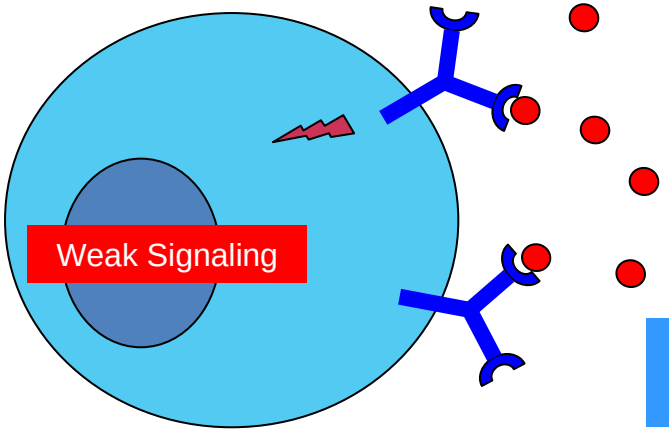


VLP (µg)	2.5	2.5	2.5	2.5	2.5
SA-TNF-α (µg)	7.5	5	2.5	1.25	0.5
Ratio SA:TNF-α GMT	**2**	**16**	**64**	**20**	**12**

Chackerian et al., J Immunol 2002

Influence of Antigen Valency on B cell Responsiveness

Poorly organized/
Monomeric self -antigen

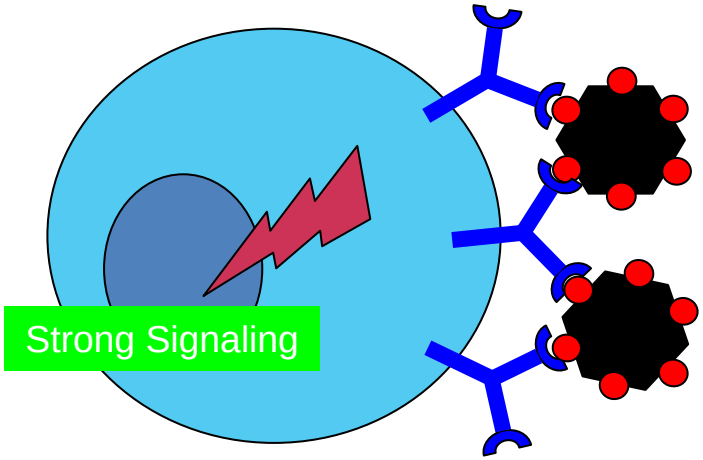


WEAK

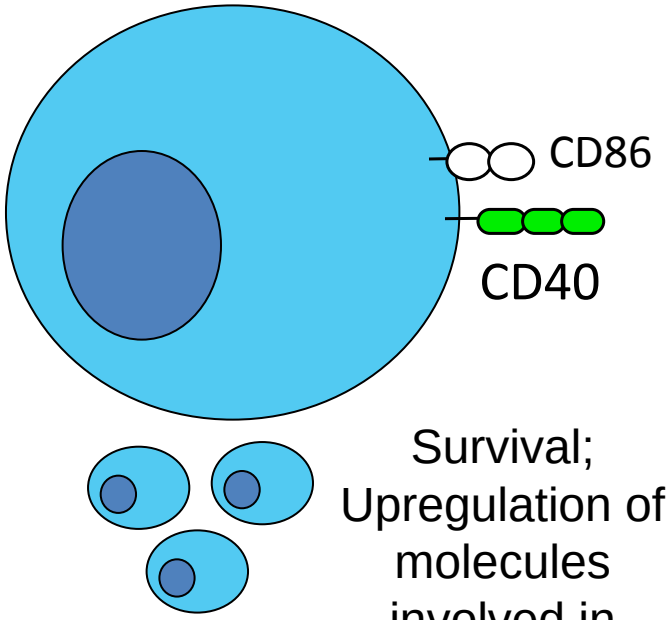
Strong adjuvants &
High Doses required

Highly repetitive
multivalent self-antigen

Adjuvants not required
Immunogenic at low doses



STRONG



Survival;
Upregulation of
molecules
involved in
interactions with
T cells

Chemical Conjugation to PV VLPs: Summary

- The surface chemistry of PV VLPs precludes chemical conjugation using the SMPH approach developed by Cytos Biotechnology.
- However, Merck has made maleimide-activated HPV VLPs and used the chemical conjugation approach to conjugate peptides derived from Influenza virus M2 (Ionescu et al., J Pharm Sci 2006).
- Diverse target antigens can be linked to VLPs via this approach.
- Conjugated VLPs can induce high titer antibody responses against self- and non-self targets, even in the absence of adjuvant.
- The magnitude of antibody responses is correlated to antigen density on the surface of the VLPs. High density antigen display is particularly required when targeting self-antigens.

Papillomavirus VLPs as a Platform for Antigenic Display

Strengths:
FDA approved platform
Can utilize diverse PV types, including animal papillomaviruses
Highly immunogenic

Weaknesses:
Chemical conjugation approaches are complicated by surface chemistry of VLPs
Lack of comprehensive data regarding genetic insertion into L1
Preexisting immunity to HPVs may preclude their use as platforms