

Advantages of Peptide Vaccines

- **Chemical purity/safety**
- **Ease of production/low cost**
- **Precisely defined and targeted T & B cell epitopes**
 - **Target cryptic epitopes**
 - **Circumvent enhancing antibody sites**
 - **Avoid T cell tolerance**
- **Stability**
- **Mutability**

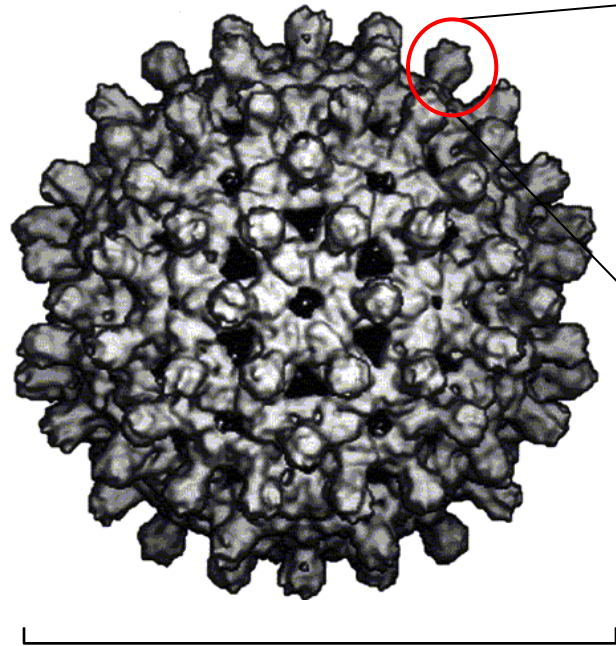
Advantages to the Use of rVLPs as Vaccine Platforms

- **Carriers for hapten and carbohydrate B cell epitopes**
- **Immunogenicity/inherent adjuvant activity**
- **Self-assembly/multimeric**
- **Safe/non-infectious**
- **High yield/low costs**
- **Therapeutic vaccinations/circumvent self tolerance
(Mab therapy alternative)**
- **Chemical conjugate vaccines**
- **Encapsidate molecular adjuvants (TLR ligands)**

Licensed rVLP Vaccines

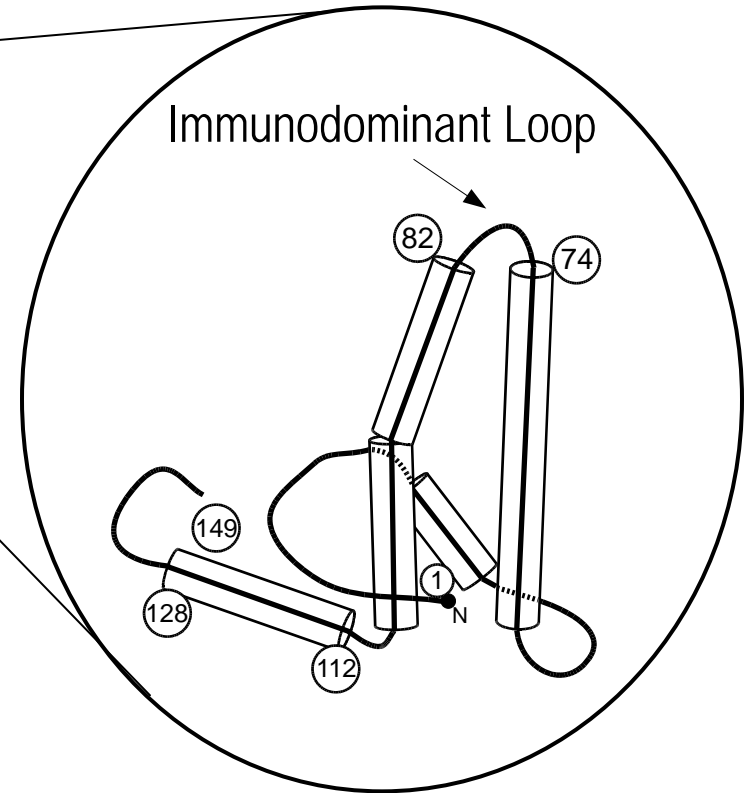
- **HBV, rHBsAg (env)**
- **HPV, L1 capsid**

HBcAg Structure



27 nm

Cryo-EM of HBcAg (7.4Å)



Polypeptide fold of monomer,
 α -helices shown.

21kDa

240 polypeptides per particle.

Licensed Hybrid-VLP Vaccines

- None

Problems:

- 1) Pre-existing HBcAg Immunity: Antibody / T cell Tolerance
- 2) Assembly/Stability (<50% success for all hybrid-VLPs)

Strategy to Address Pre-existing Immunity Problem: Selection of a Species Variant Core Particle

Hepadnavirus Family

Genus: *Orthohepadnavirus*

Primates: human, non-human

Rodents: woodchuck, ground squirrel, arctic squirrel

Genus: *Avihepadnavirus*

Duck

Heron

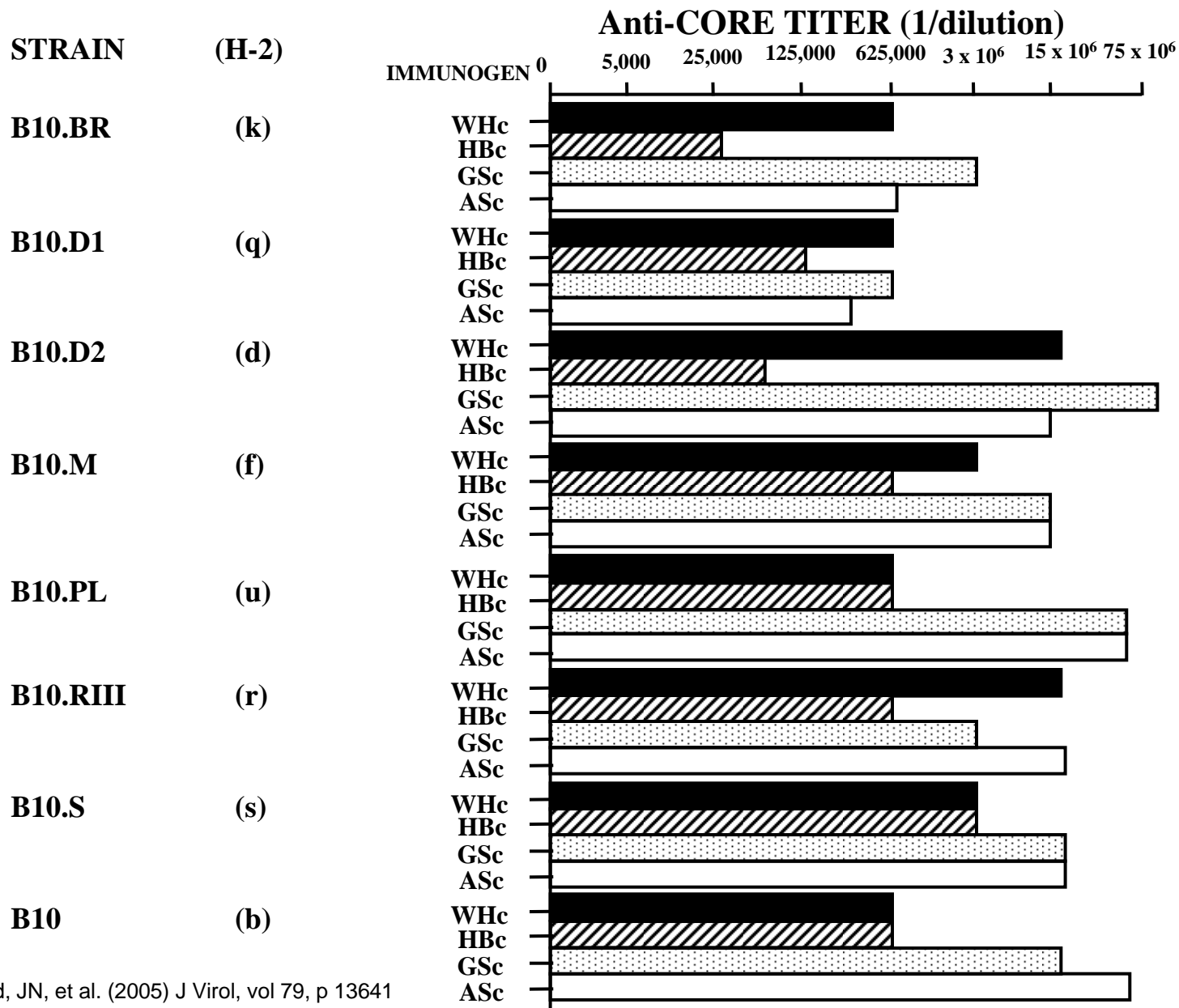
Goose

Characteristics: hepatotropic

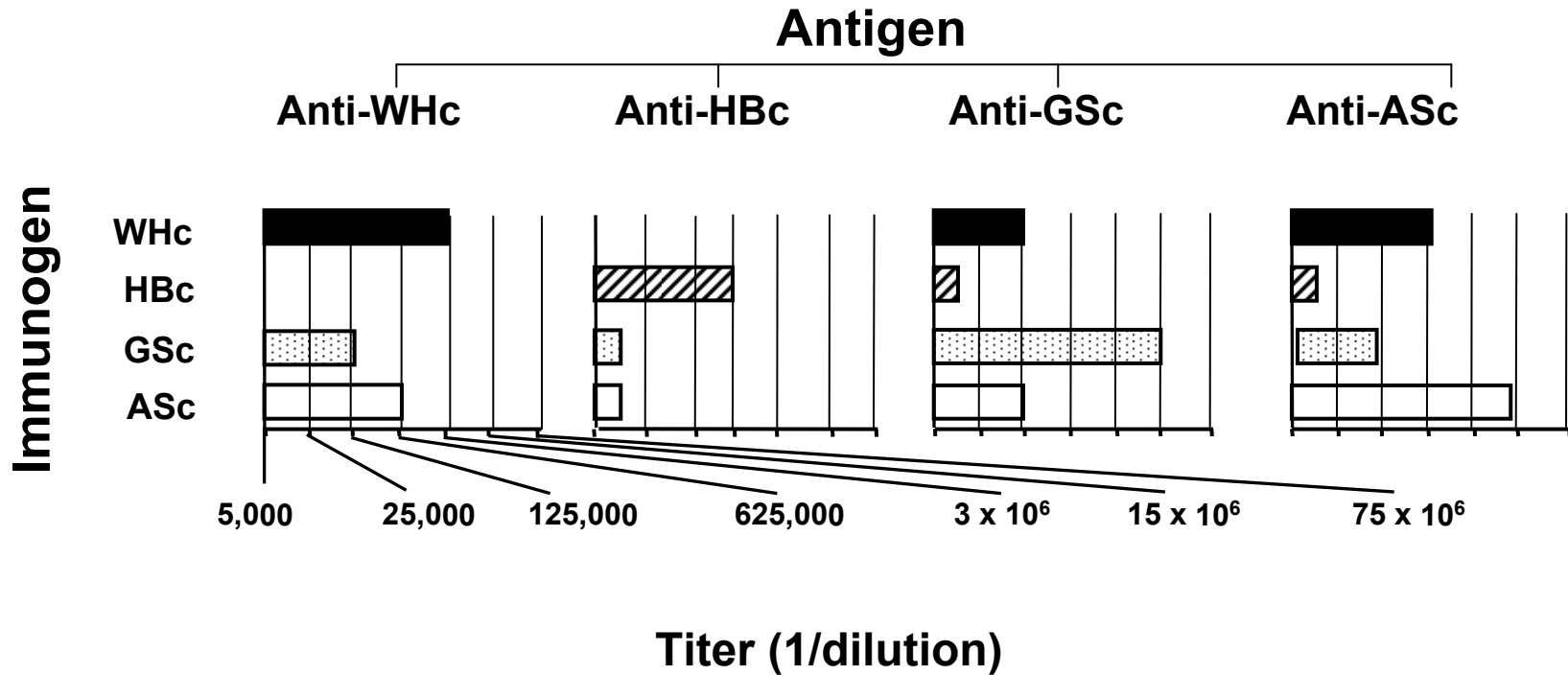
partially dsDNA

RT of RNA intermediate

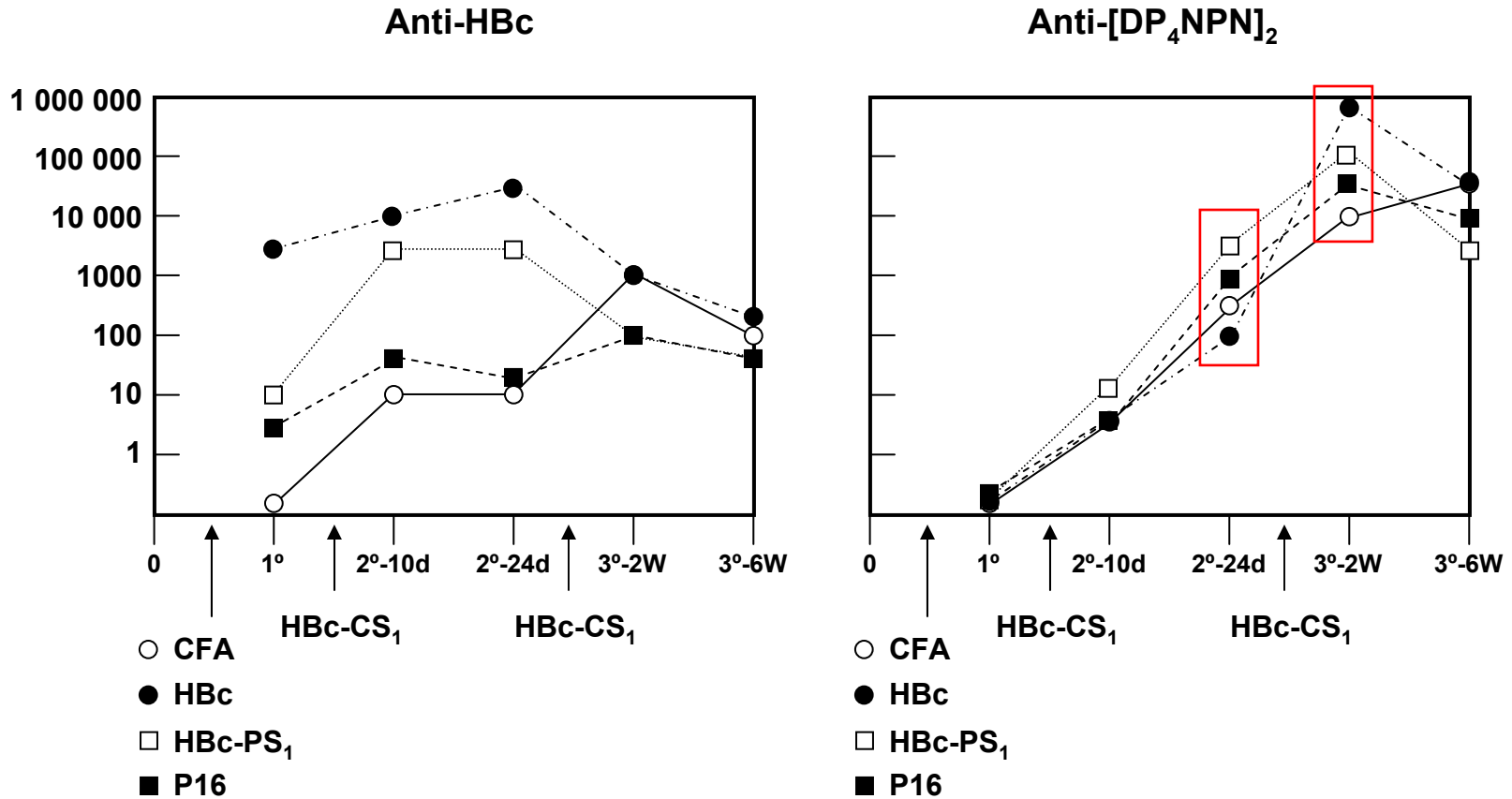
Rodent Cores Are More Immunogenic than HBc



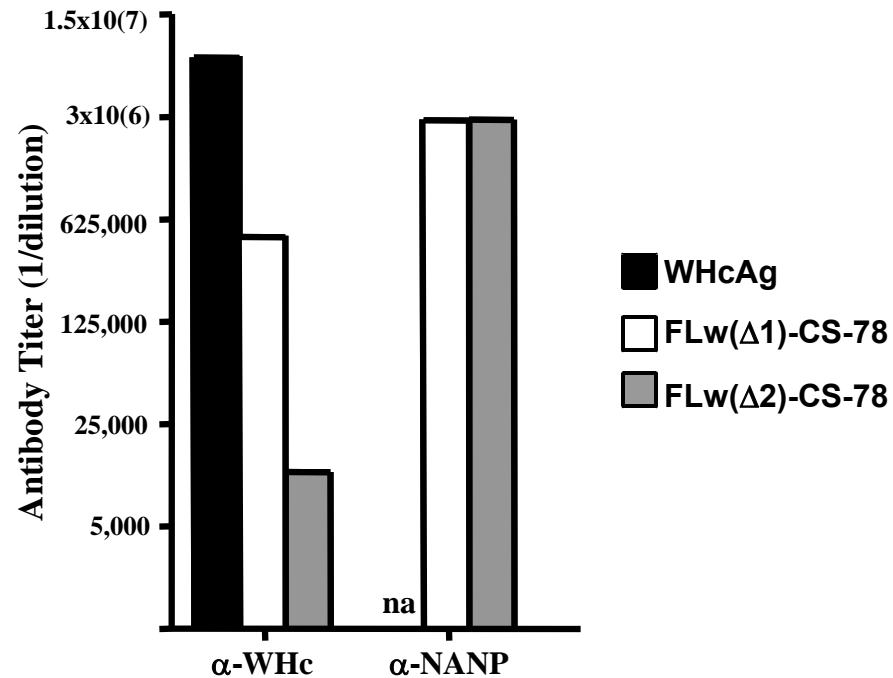
HBc and WHc Do Not Cross-React at the Ab Level



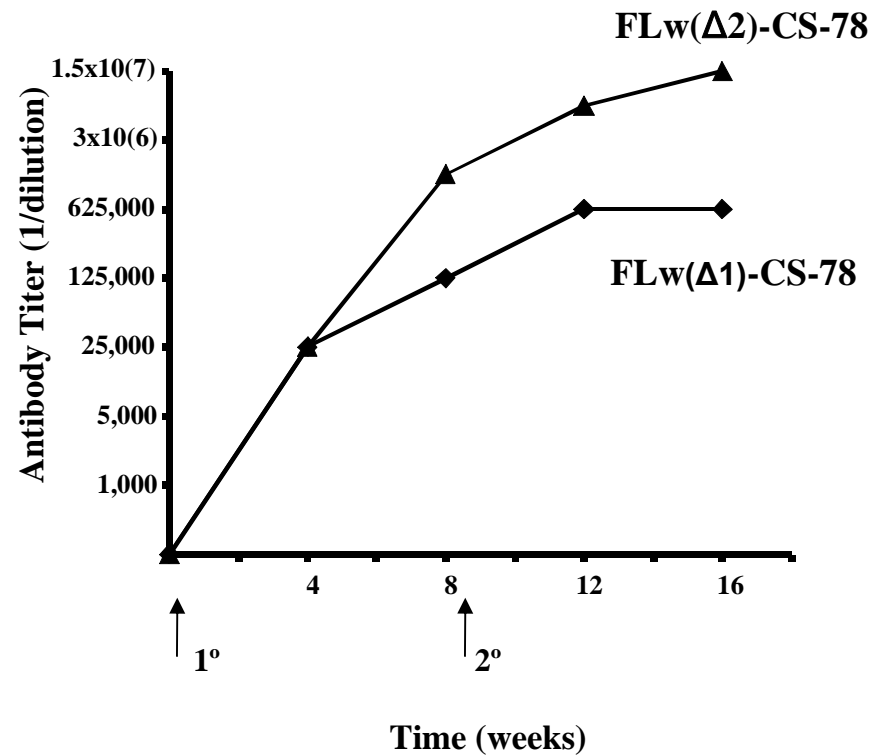
Effect of Pre-existing Antibody to HBcAg Carrier



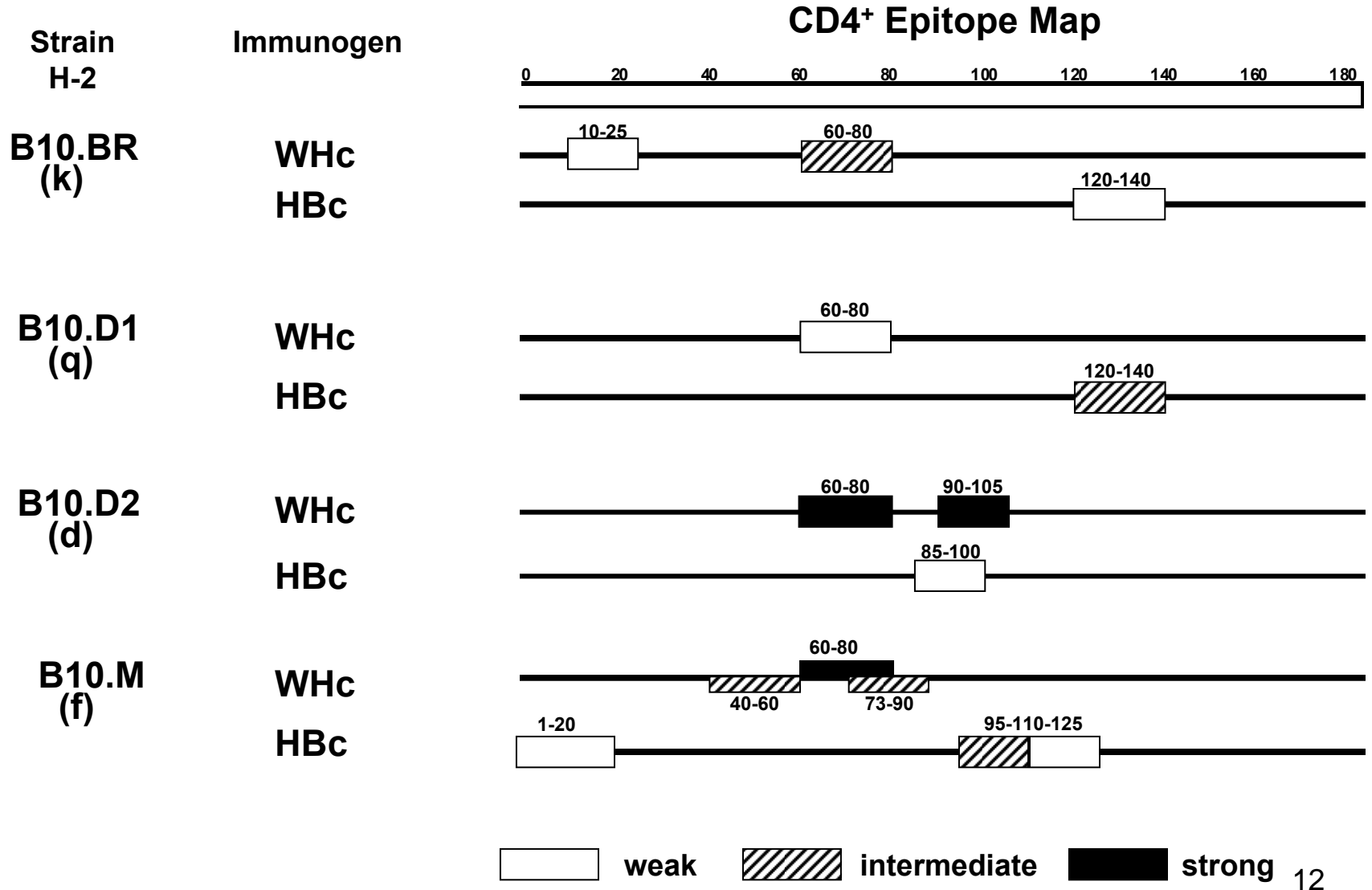
Deletion of VLP Carrier B cell Epitopes Lowers Carrier Antigenicity



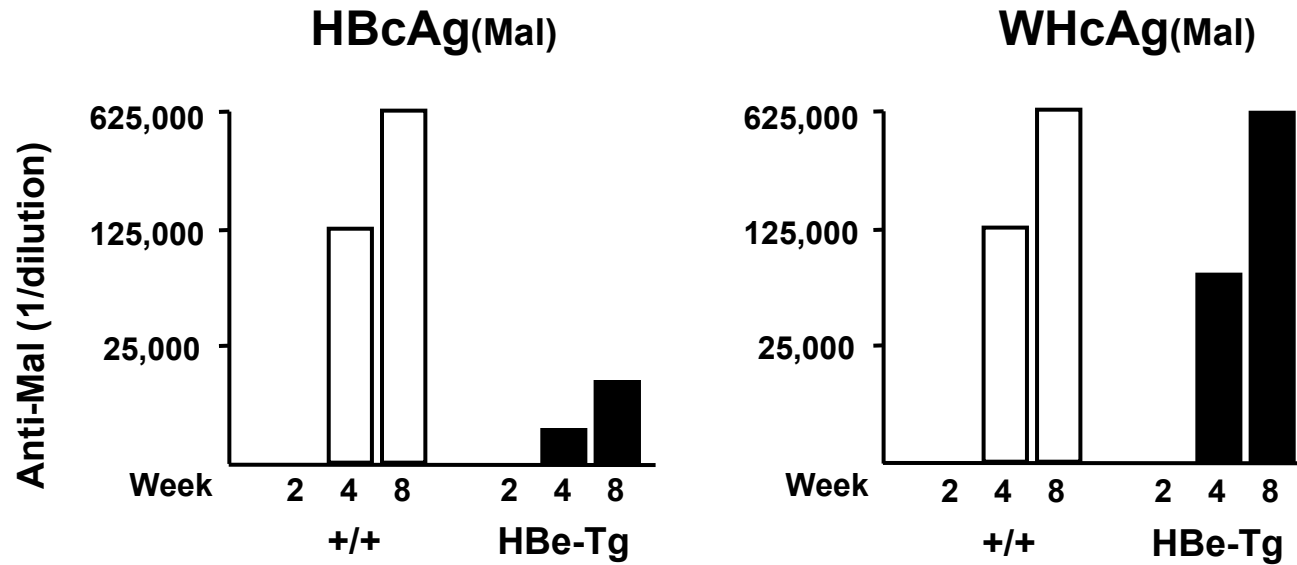
Deletion of Carrier B cell Epitopes Improves Insert Immunogenicity



Low Level Cross-Reactivity Between WHc and HBc at CD4 T cell Level

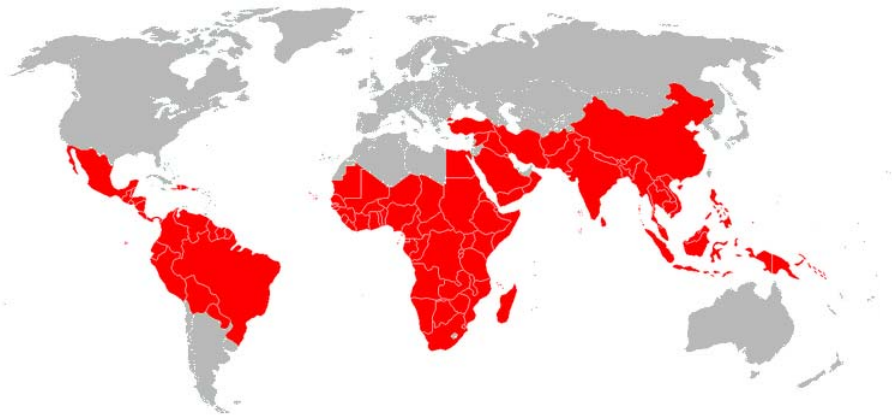


Use of WHc Circumvents T cell Tolerance to the HBc



Endemic Regions of Malaria & HBV

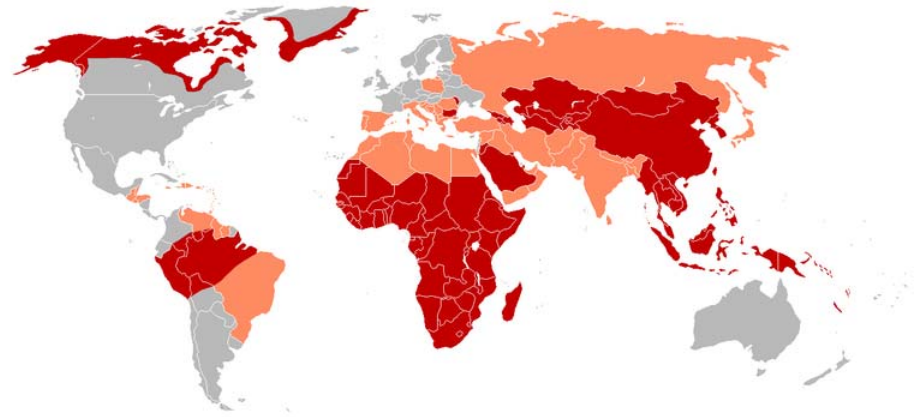
Malaria



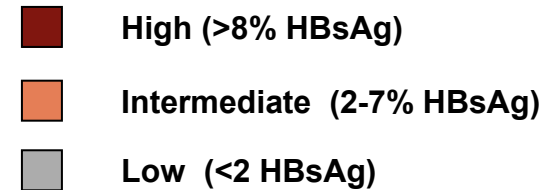
Presence of Malaria



HBV



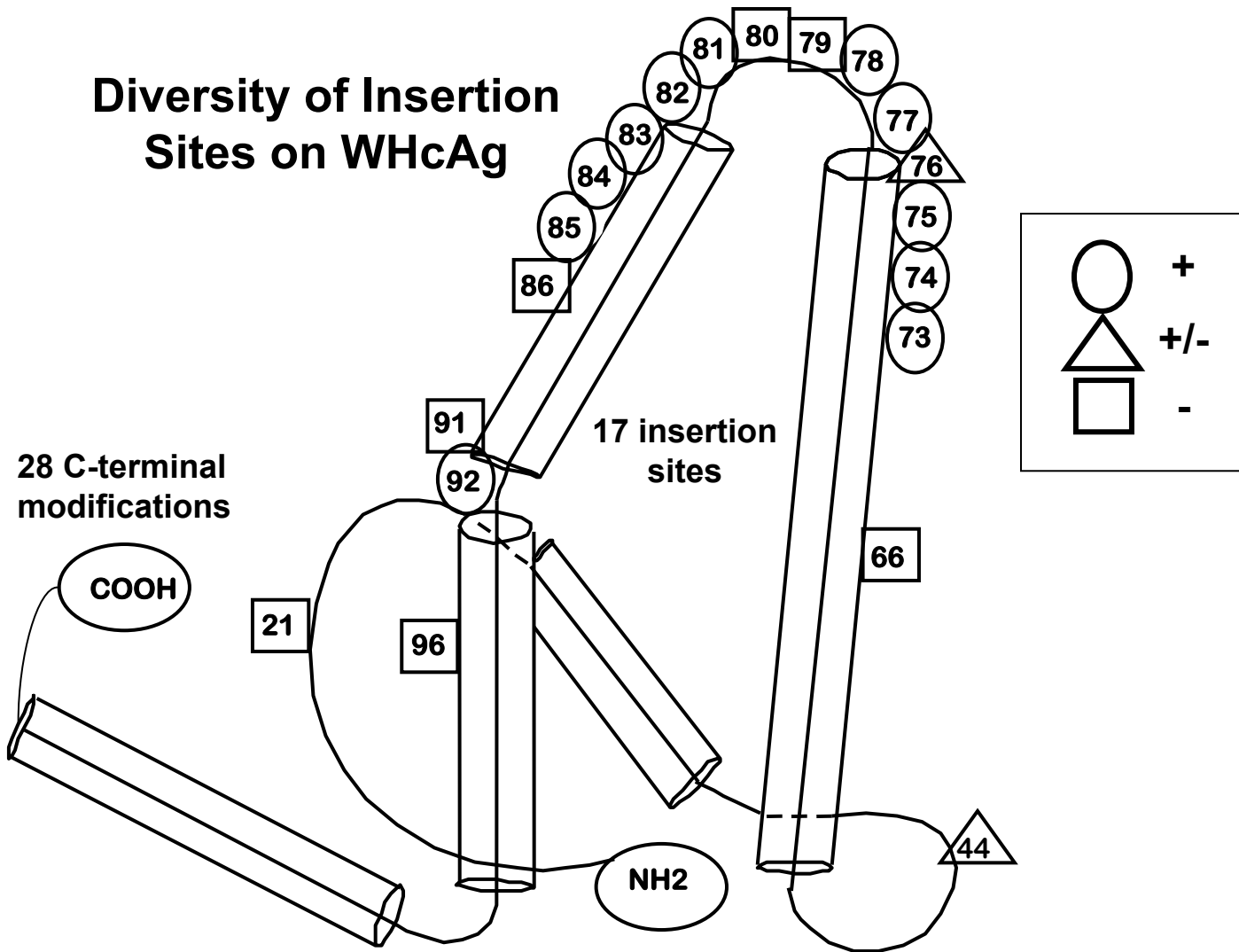
HBV Carrier Rates



http://www.cdc.gov/malaria/distribution_epi/distribution.htm

<http://wwwnc.cdc.gov/travel/yellowbook/2010/chapter-2/hepatitis-b.aspx>

Strategy to Address Hybrid VLP Assembly Problem



















Diversity of C-termini on WHcAg

Combinatorial Technology

















Optimal Assembly Platform

Epitope	C-terminus	Insert Position
Mal fal.	C-long	78
Mal vivax	HyW	78
Cetp	HyW	74
FV-1	HyW2	75
FV-2	150C	74
HIV-2	HyW2	75
HIV-3	HyW2	75
HIV-4	150C	75
FluA M2	WT-R	74
FluA M2(-)	HyW	78
HCV-E2 #17	HyW2	75
HCV-E2 #24	HyW2	78

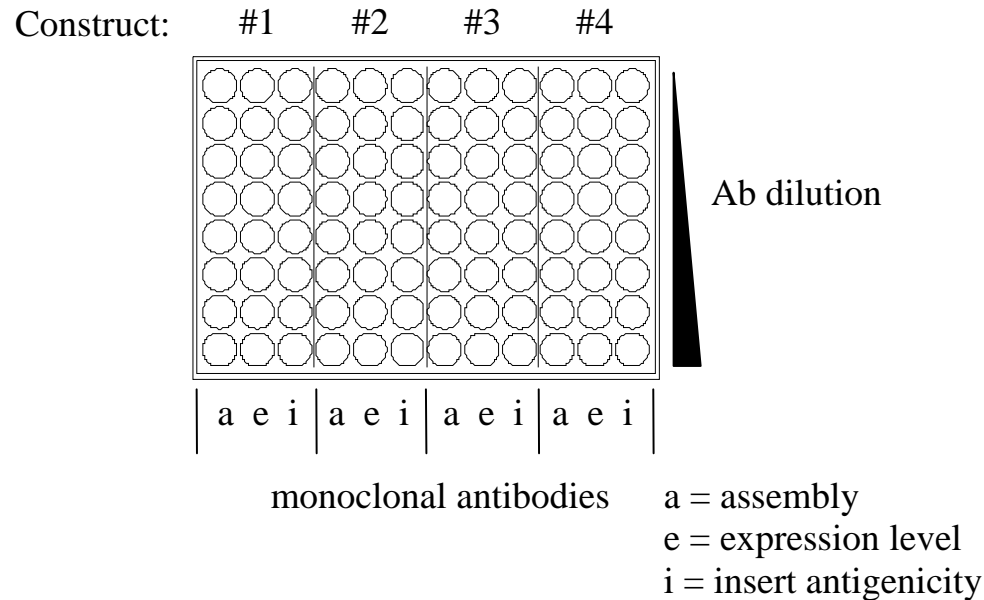
Basic Inserts (pI >7) Adversely Affect Assembly of Hybrid VLPs

Epitope	Sequence	pI	VLP Assembly
AZ2	FRHDSGY	7.5	
OMP-2	EDANGTRDHKKGRHT	9.5	
HIV4.1	RIKQIGMPGGK	11.8	
IgE 413-435	GETYQSRVTHPHLPRALMRSTTK	11.4	
HV1	GEIKNCSFNISTSIRGKVQKEYAFF	9.4	
HV3	PKVSFEPIPIHYCAPAGFAILKCNN	8.2	
SEB	KKKVTAQELD	9.4	
HV2	LTSCNTSVITQACPKVSFEPIPIHYC	7.2	
HV4	THGIRPVVSTQLLLNGSLAEEE	4.5	
MV	DRAAGQPAGDRADGQPAG	4.3	
CE	FGFPEHLLVDFLQSL	4.2	
AZ1	DAEFRHDSGYEV	4.1	
M2(-)	SLLTEVETPIRNEWGARANDSSD	4.0	
HC10	[Proprietary]	3.4	
Mal	NANPNVDPNANPNANPNANP	3.8	
MB	DPPPPNPNDPPPPNPN	3.5	

Acidification of the Insert Rescues Assembly

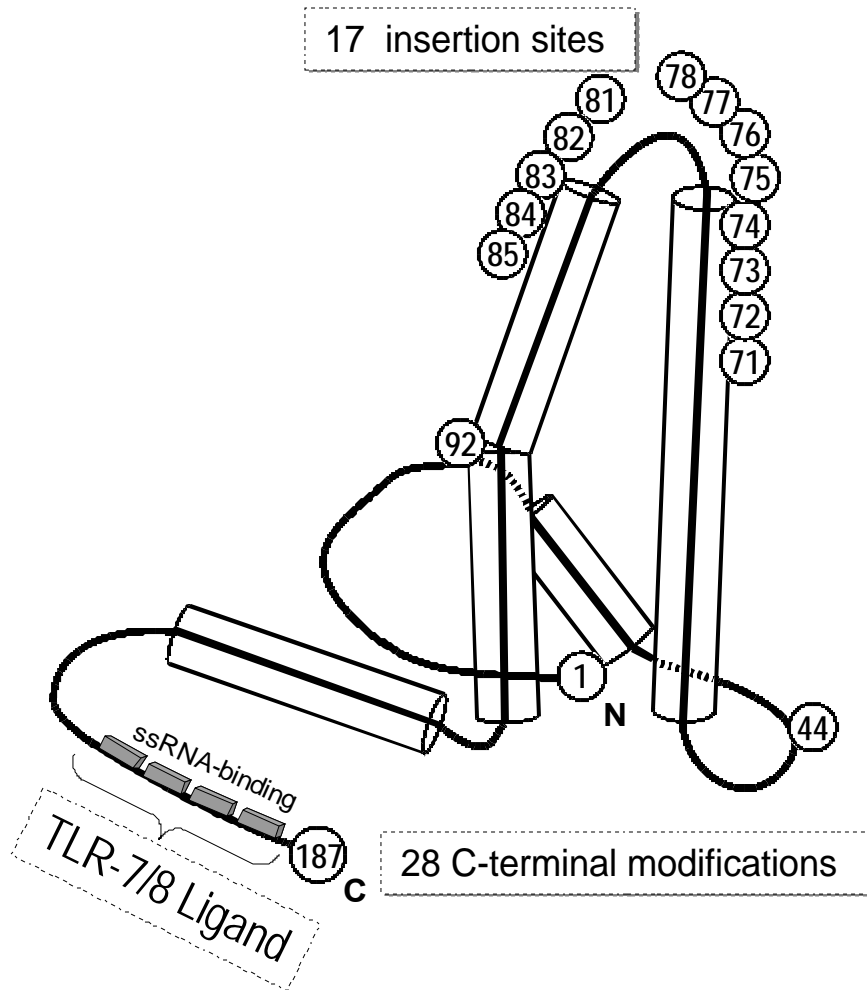
	Epitope	Sequence	pI	VLP Assembly
WHcAg Platform	AZ2	FRHDSGY	7.5	
		<u>EE</u> FRHDSGY <u>EE</u>	4.0	
	OMP-2	EDANGTRDHKKGRHT	9.5	
		<u>EE</u> EDANGTRDHKKGRHT <u>EE</u>	4.8	
	HIV4.1	RIKQIGMPGGK	11.8	
		<u>EE</u> RIKQIGMPGGK <u>EE</u>	4.6	
HBcAg Platform	AZ2	FRHDSGY	7.5	
		<u>EE</u> FRHDSGY <u>EE</u>	4.0	
	OMP-2	EDANGTRDHKKGRHT	9.5	
		<u>EE</u> EDANGTRDHKKGRHT <u>EE</u>	4.8	
	HIV4.1	RIKQIGMPGGK	11.8	
		<u>EE</u> RIKQIGMPGGK <u>EE</u>	4.6	
GScAg Platform	AZ2	FRHDSGY	7.5	
		<u>EE</u> FRHDSGY <u>EE</u>	4.0	
	HIV4.1	RIKQIGMPGGK	11.8	
		<u>EE</u> RIKQIGMPGGK <u>EE</u>	4.6	

Rapid Screening Technology



- Proprietary ELISA-based method
- Applied to bacterial lysate, avoiding labor-intensive purification step
- Assesses: expression; assembly; insert antigenicity
- Facilitates rapid turn-around, i.e., epitope conception to purified VLP in 2-4 weeks

Combinatorial Tools for WHcAg



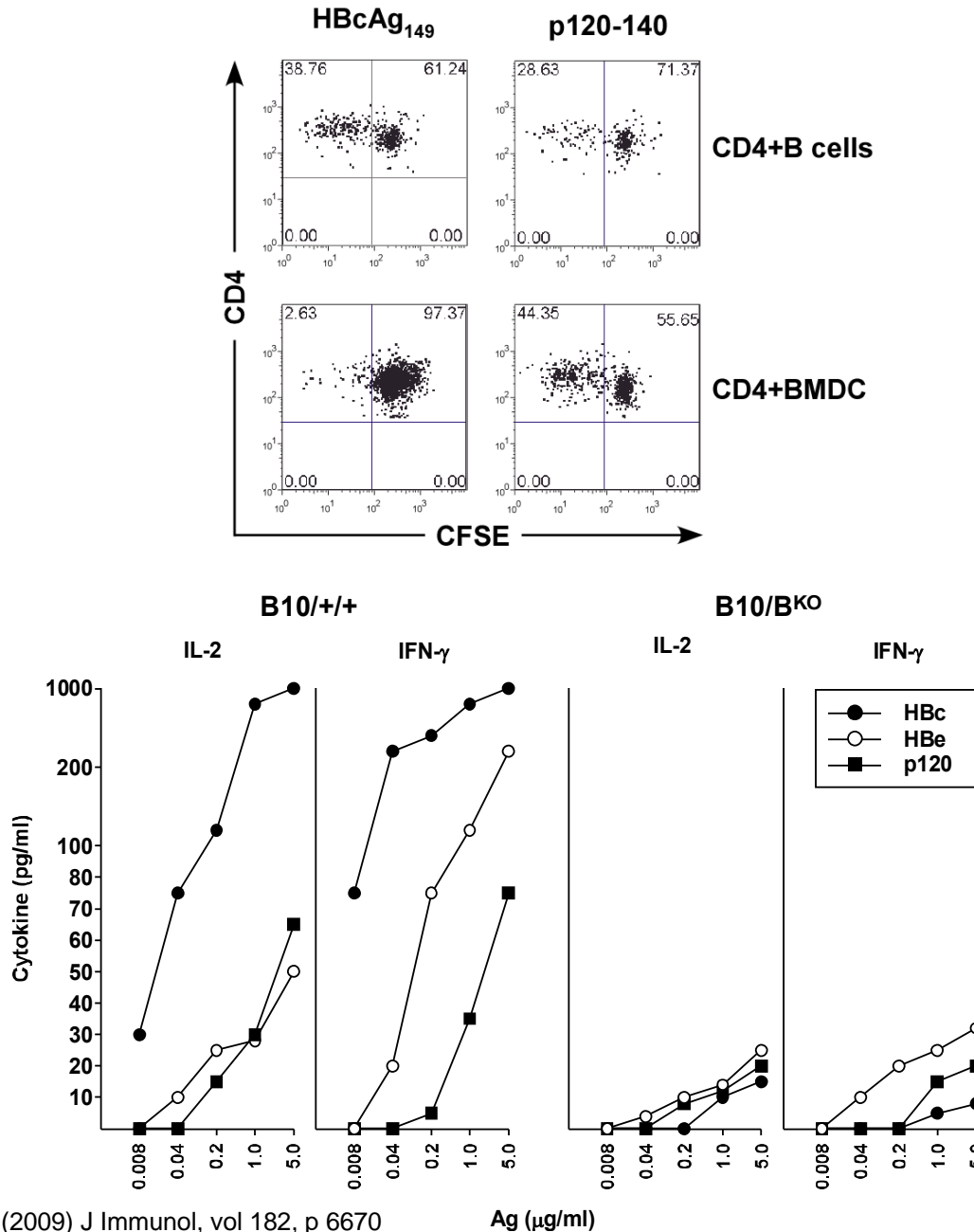
- 17 Insertion Sites
- 28 C-terminal Modifications
- Acidification of the loop
- Rapid Screening Technology

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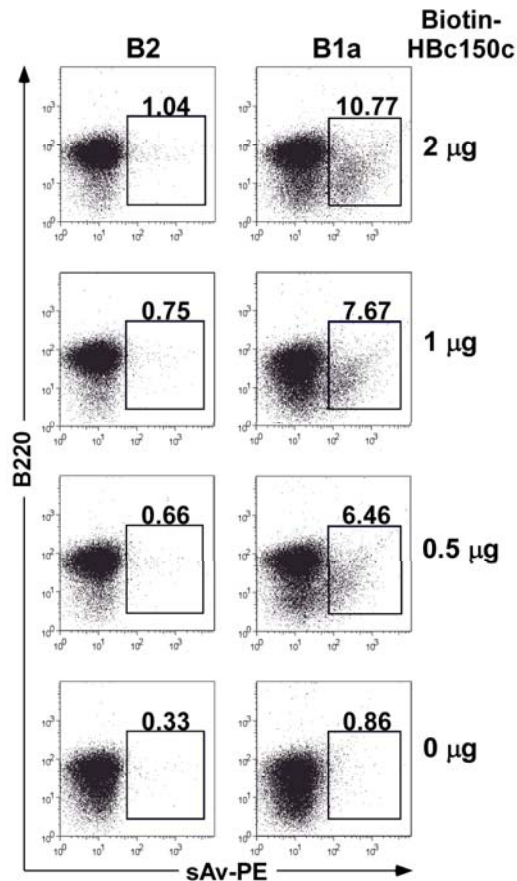
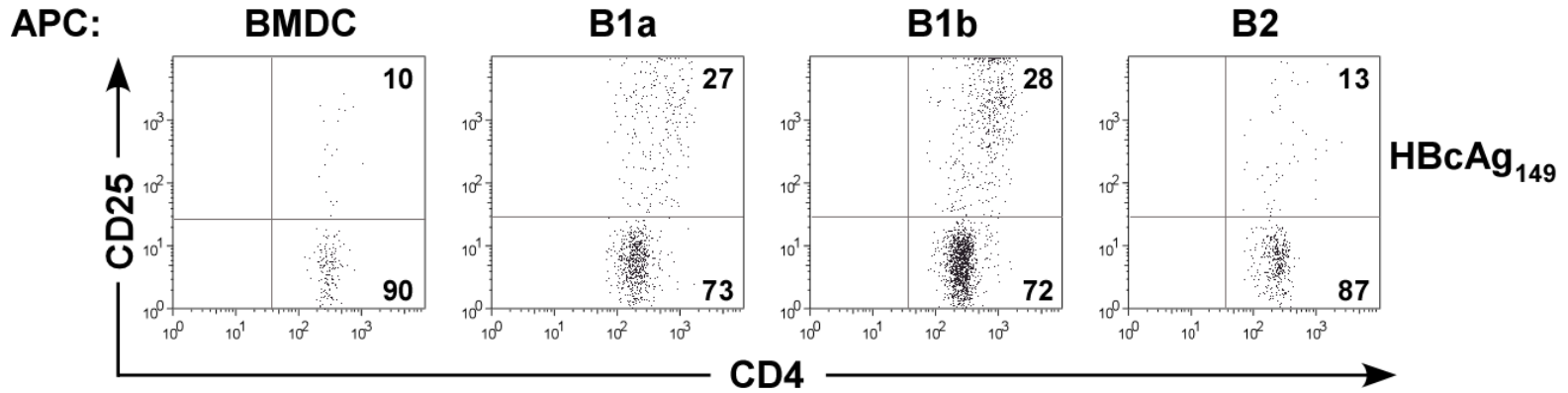
>95% Assembly Success

Billaud JN, et al., J Virol (2005) vol 79, p 13656
Billaud JN, et al., Vaccine (2007) vol 25, p 1593

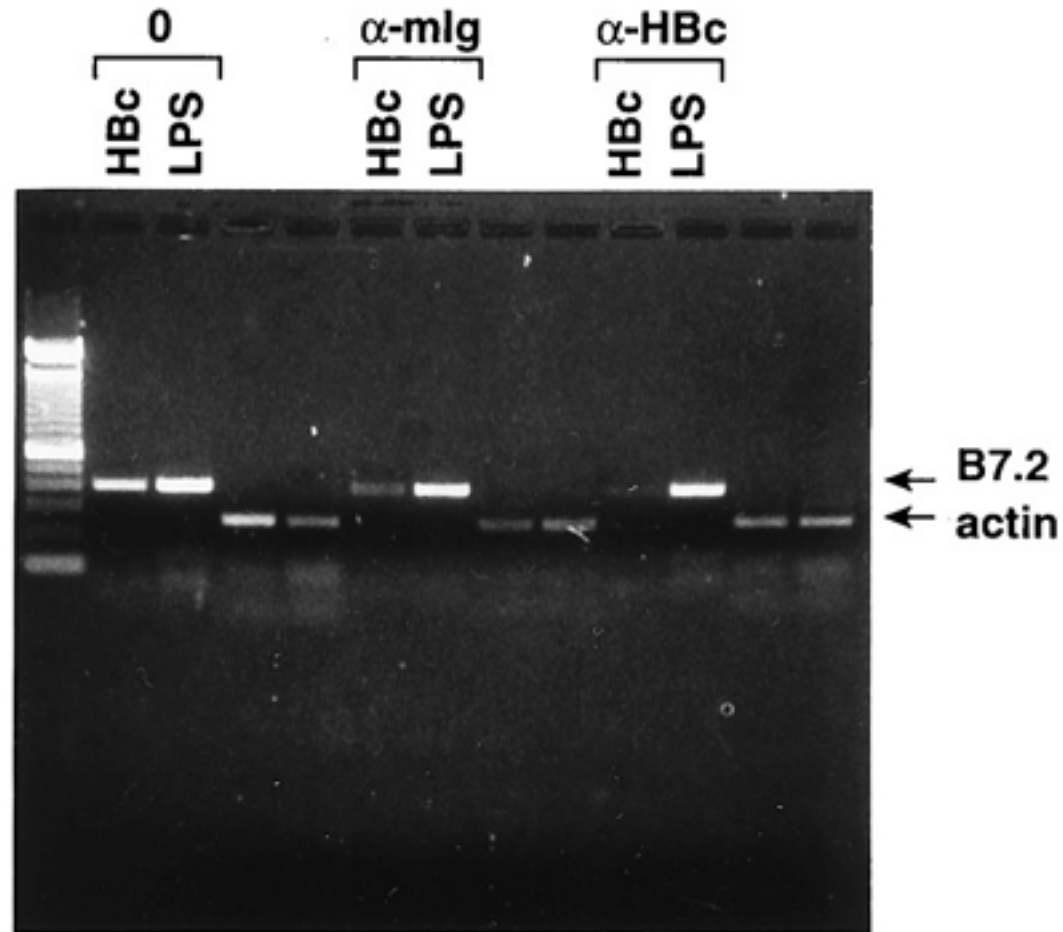
HBc/WHc Are Preferentially Presented by B cell APC *in vitro* and *in vivo*



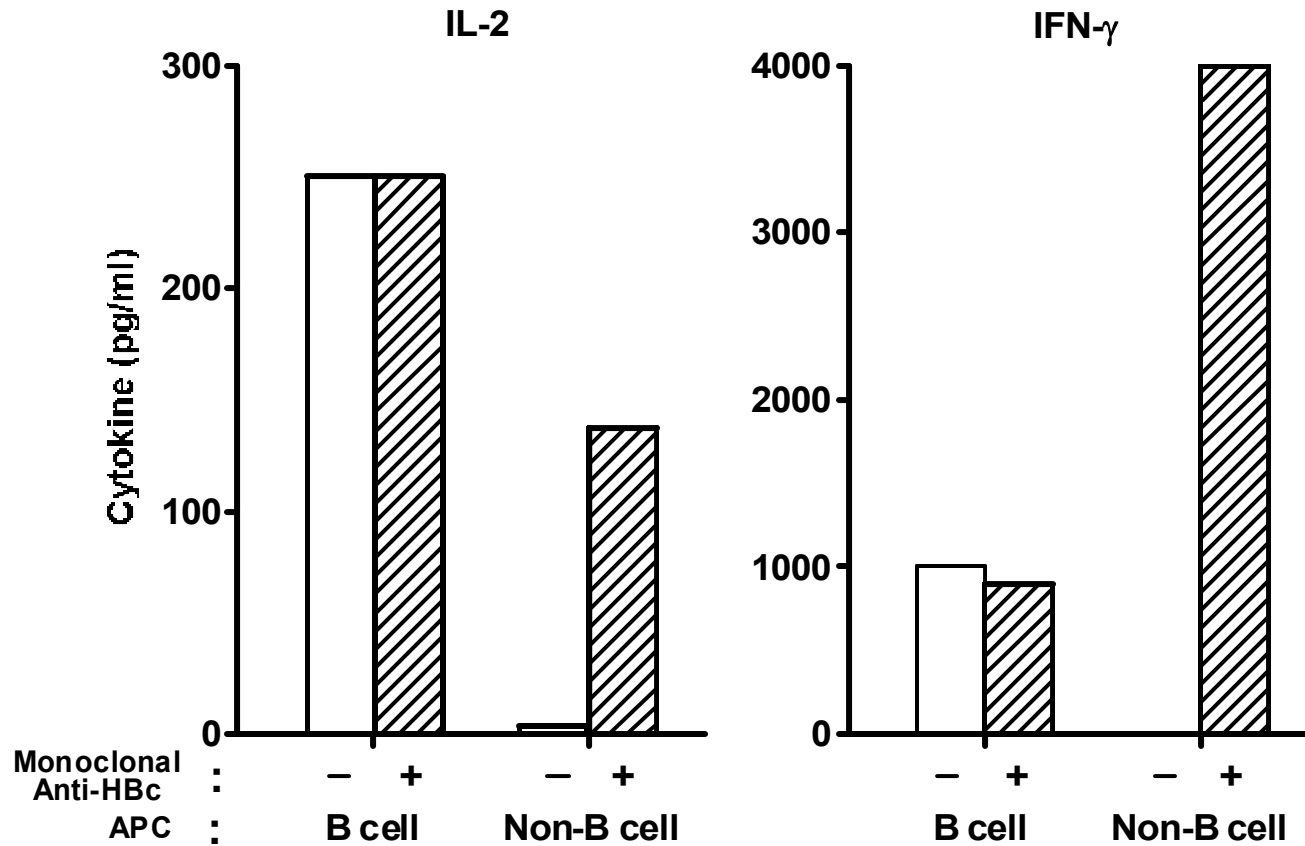
HBc Is Preferentially Presented by B1 APC



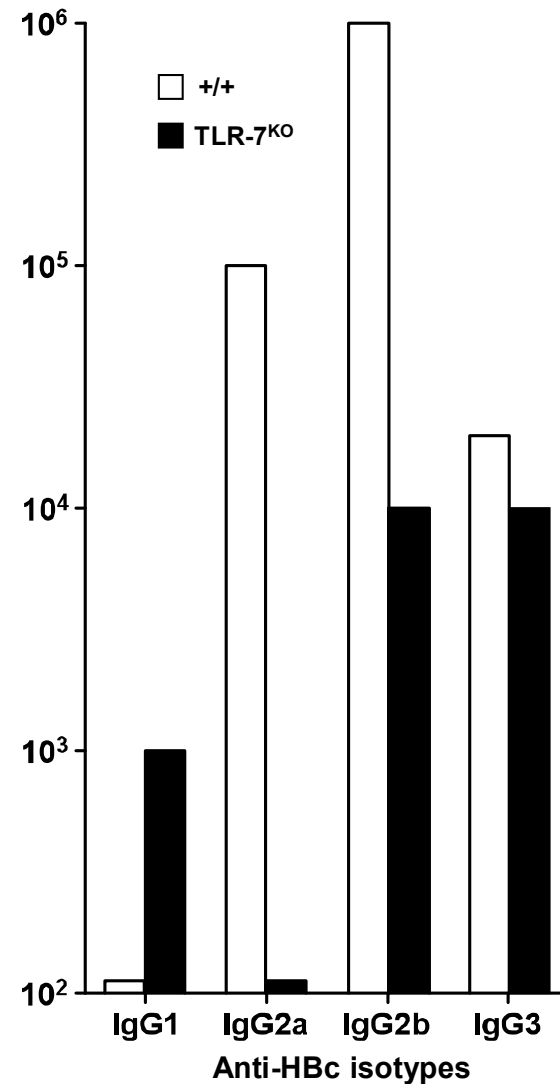
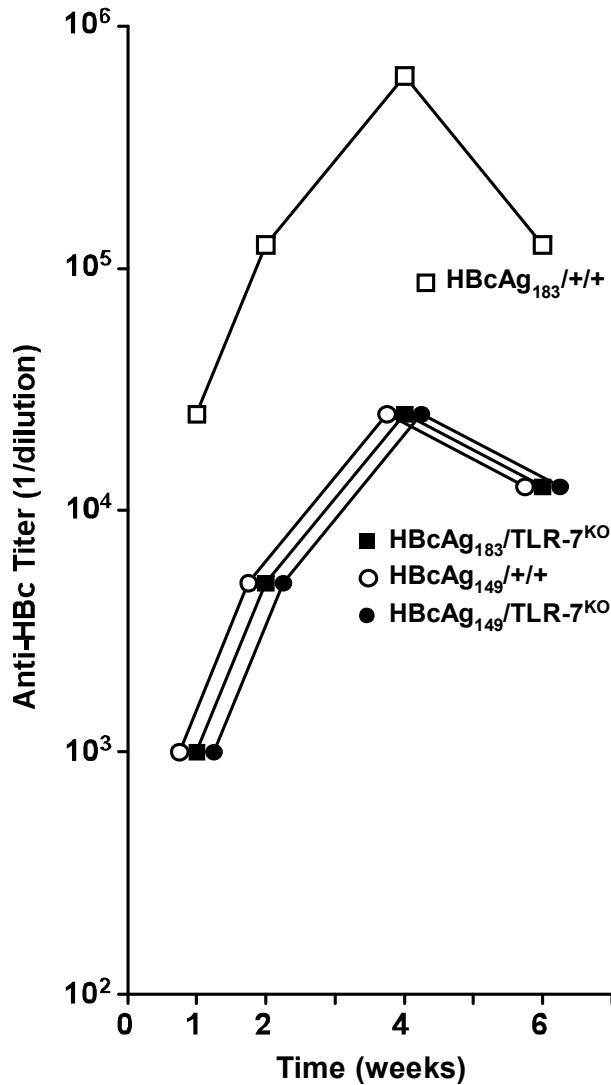
B cell Uptake of HBc Elicits Upregulation of B7.2 Costimulatory Message



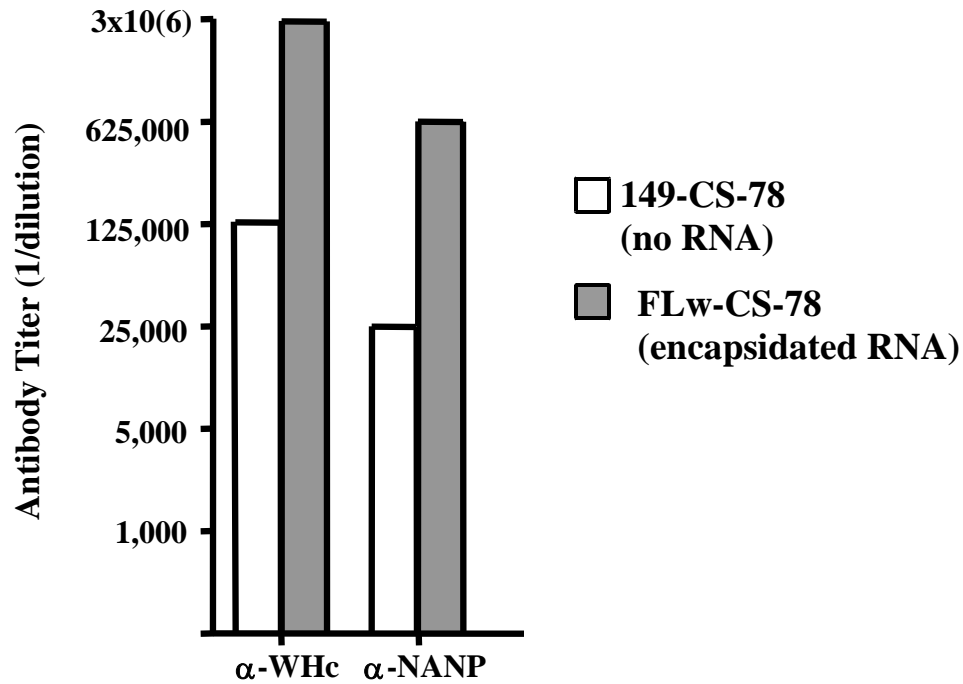
Non-B cell APCs Can Process and Present Immune-Complexed Hbc



Full Length HBc (ssRNA+) Possesses Enhanced Immunogenicity Mediated Through TLR-7 Activation

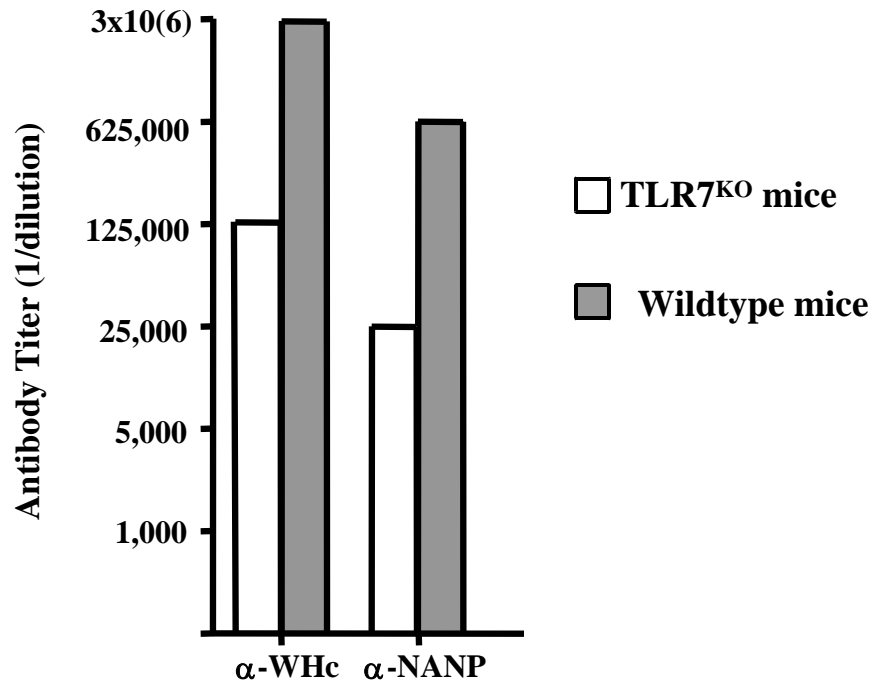


ssRNA Encapsidation Improves B Cell Response to NANP Insert

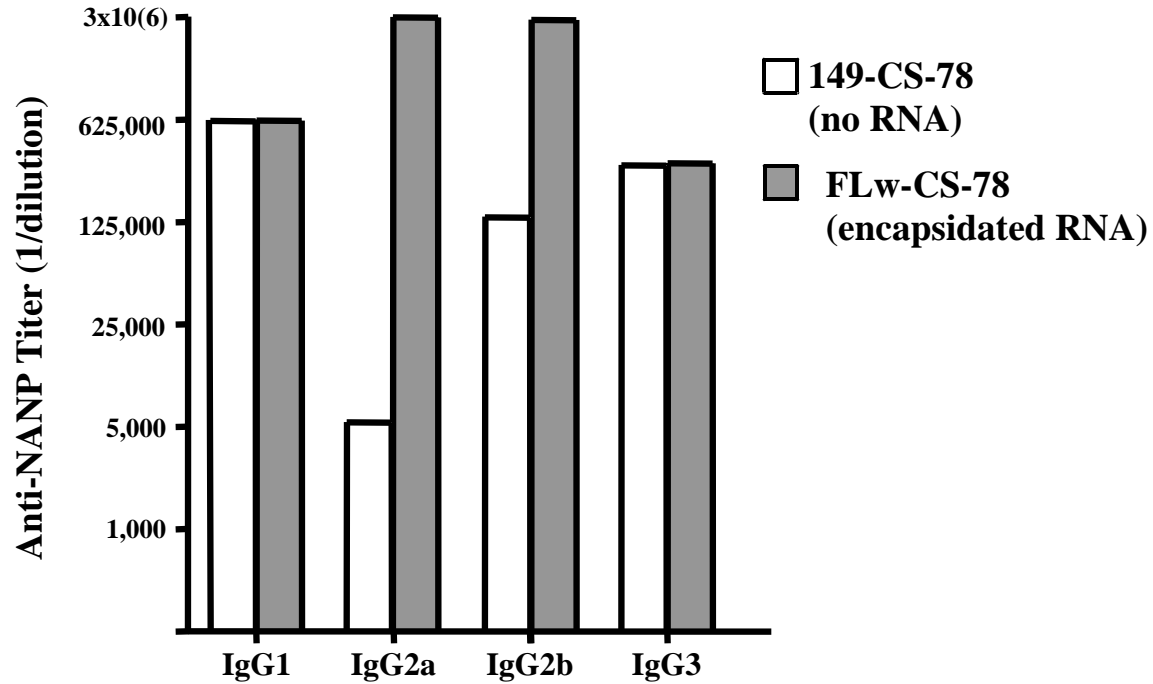


ssRNA Acts Through TLR7

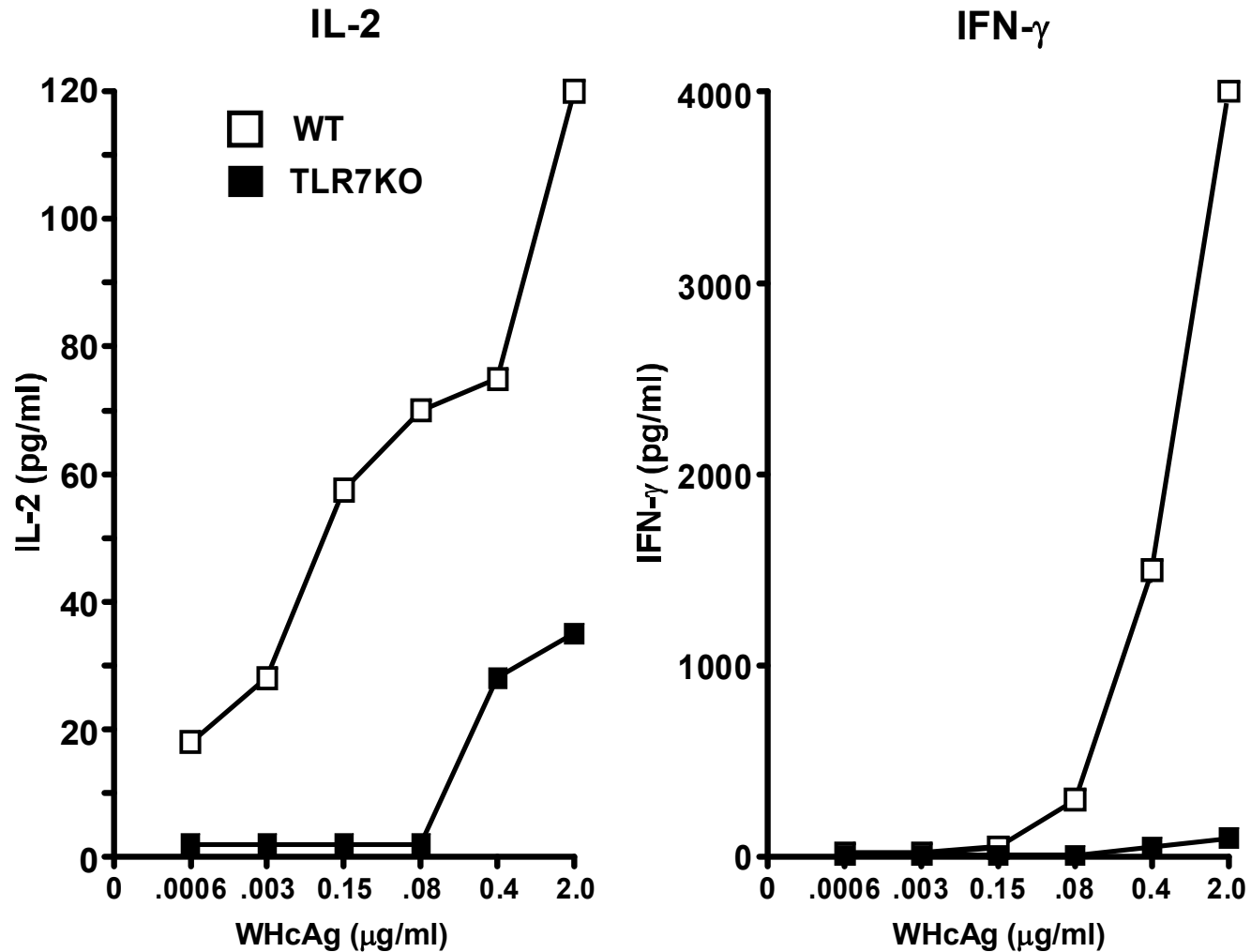
IgG Titers for Mice Immunized with FLw-CS-78 (w/ ssRNA)



Encapsulation of TLR7 Ligand Alters IgG Isotype Distribution



HBc-Specific CD4 Cells Are Regulated by TLR-7 Activation

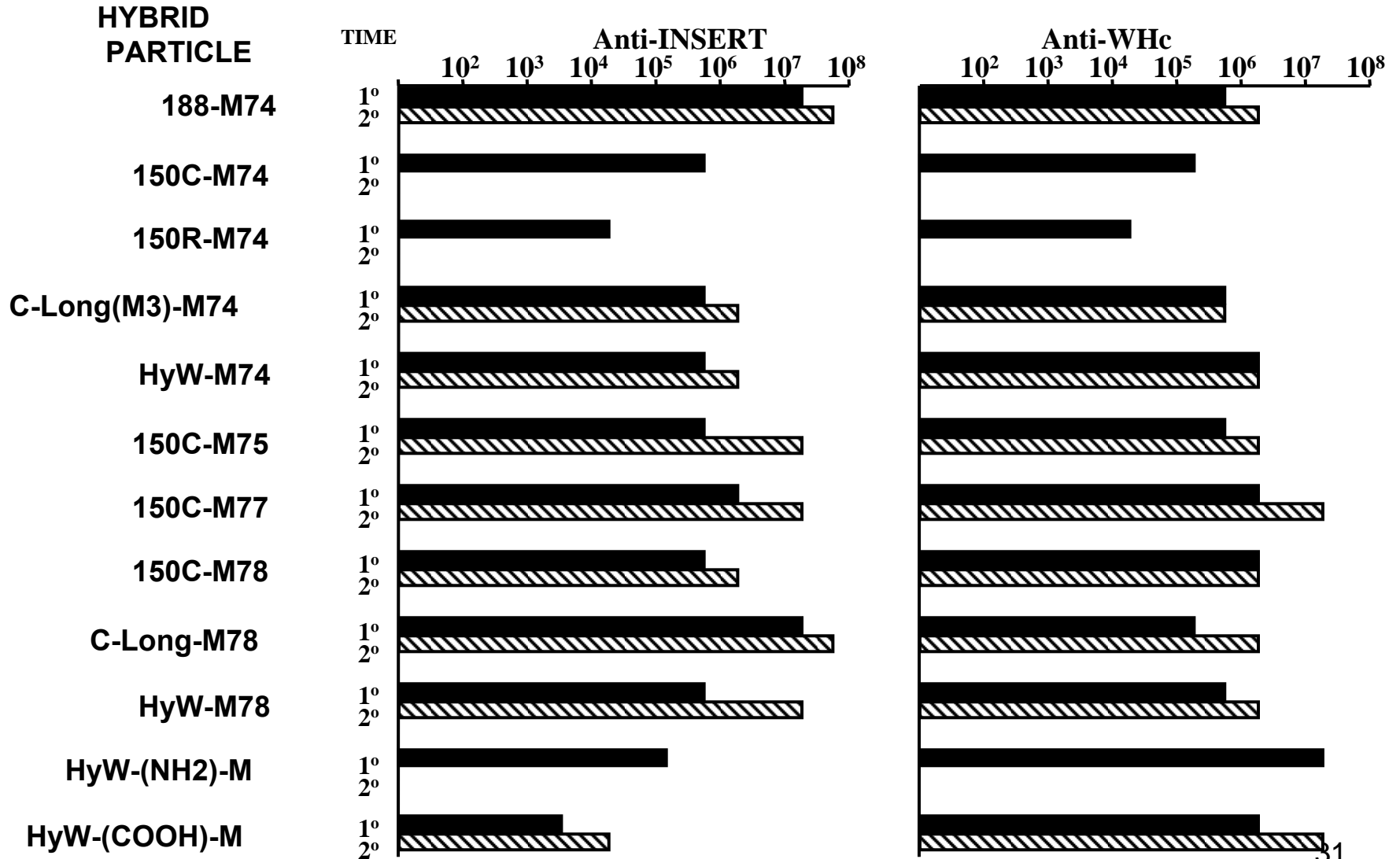


Advantages of Integral Molecular Adjuvants

- Immune stimulant is delivered directly to the APC
- Co-ligation of B cell receptor and TLR
- Avoids the potential toxicity of systemic administration
- The ligand is protected from nucleases or proteases

A Variety of Hybrid WHc-CS VLPs Have Been Produced

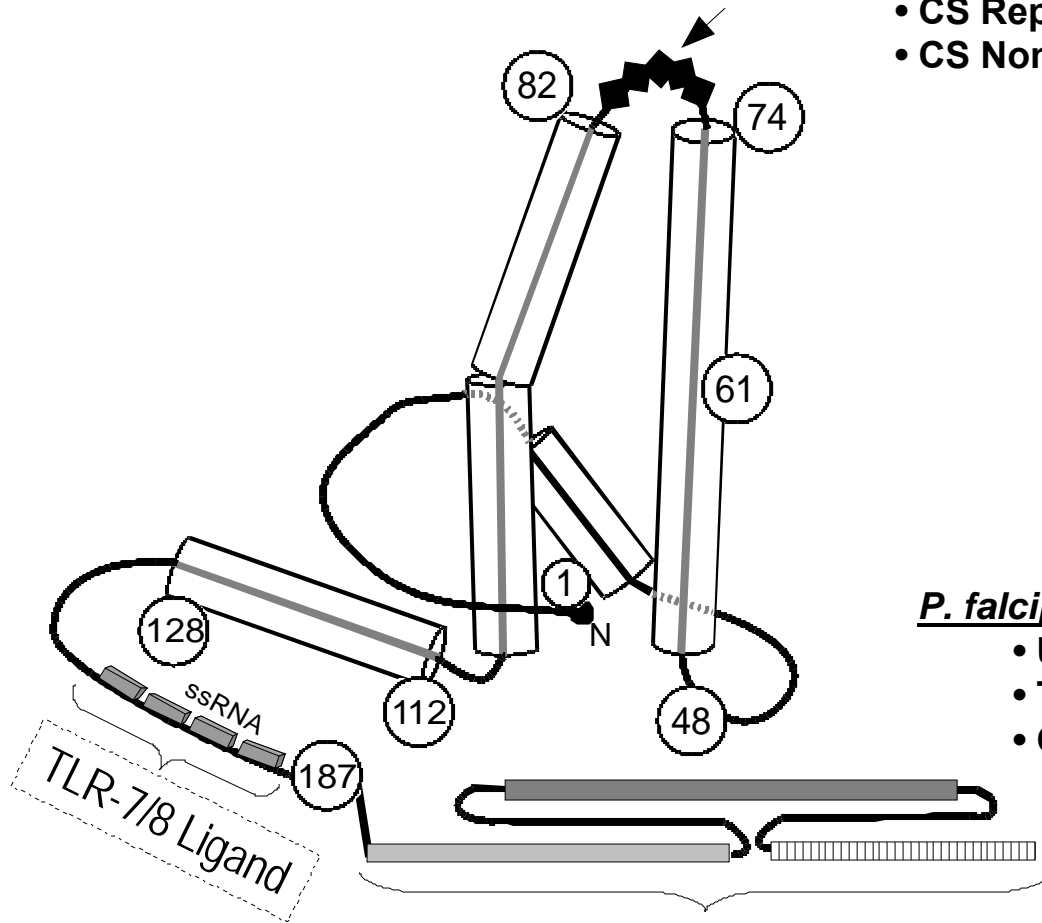
IMMUNOGENICITY



WHc-Malaria VLP Vaccine Candidates

P. falciparum B cell epitopes

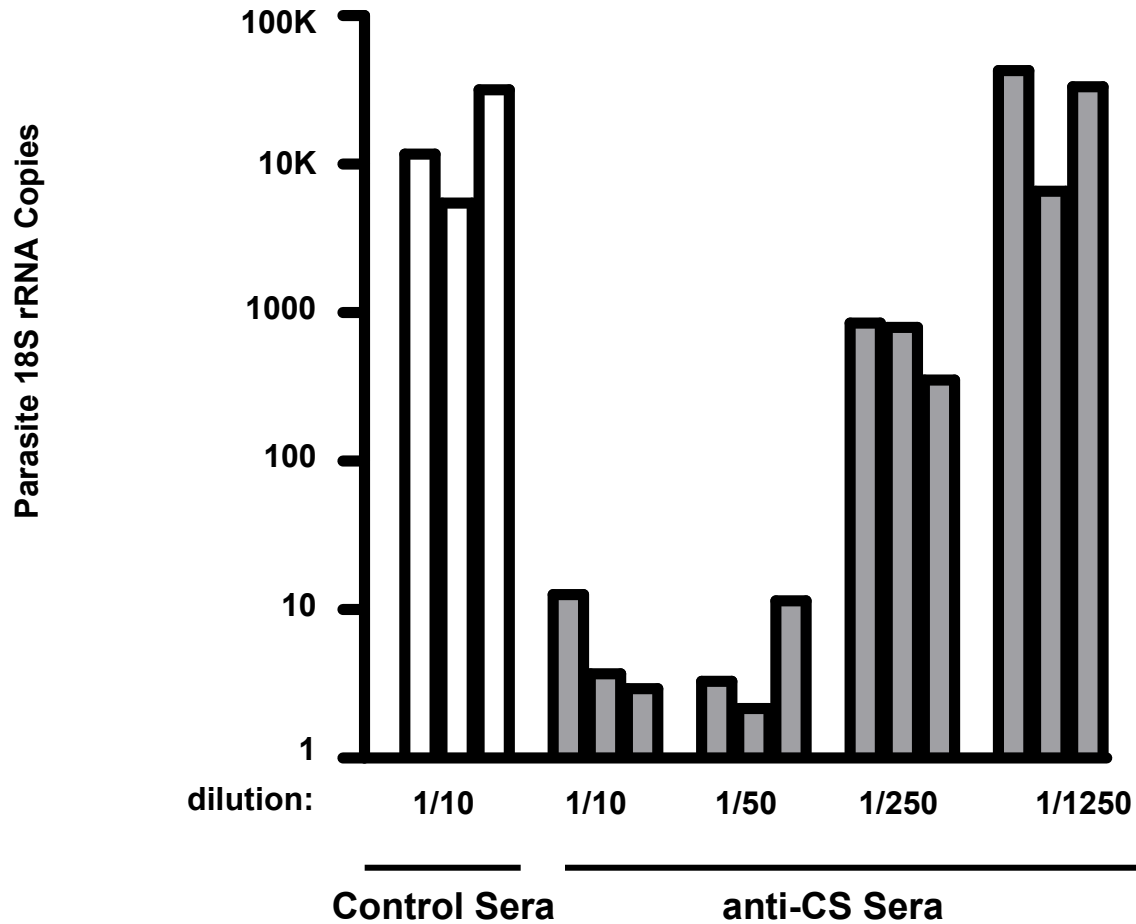
- CS Repeat (1 to 2)
- CS Non-Repeat (1 to 5)



P. falciparum CS T cell epitopes (1 to 3)

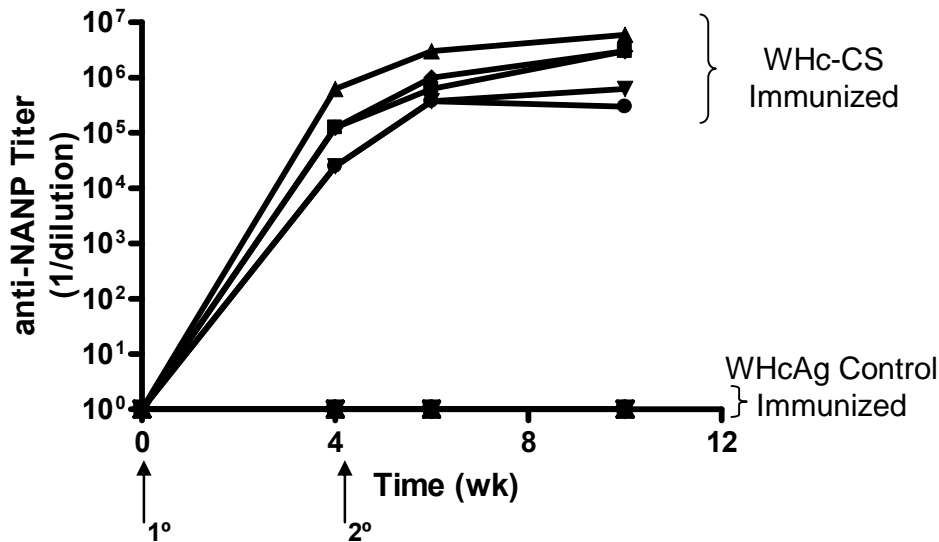
- UTC
- TH.3R
- CS.3T

Passive Transfer of Anti-WHc-CS VLP Antibodies Prevents Hybrid-Sporozoite Liver Infection

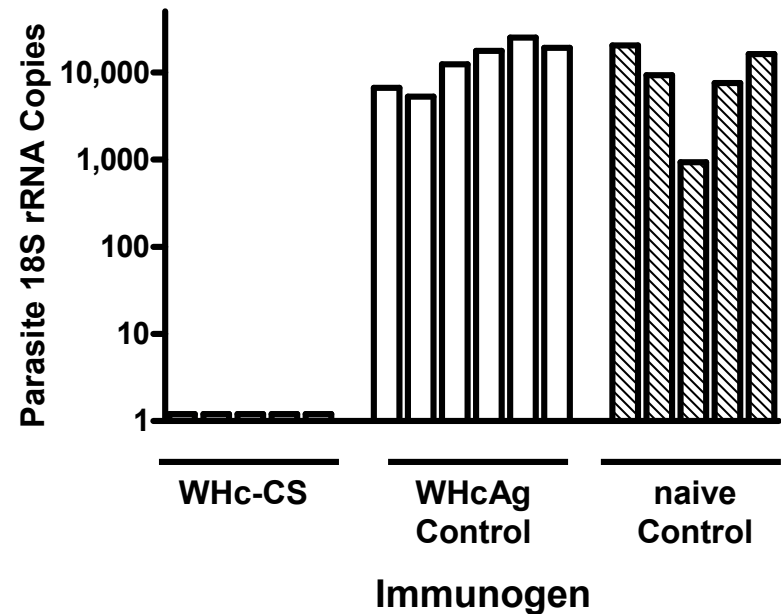


Active Immunization with WHc-CS VLP Prevents Hybrid Sporozoite Liver Infection

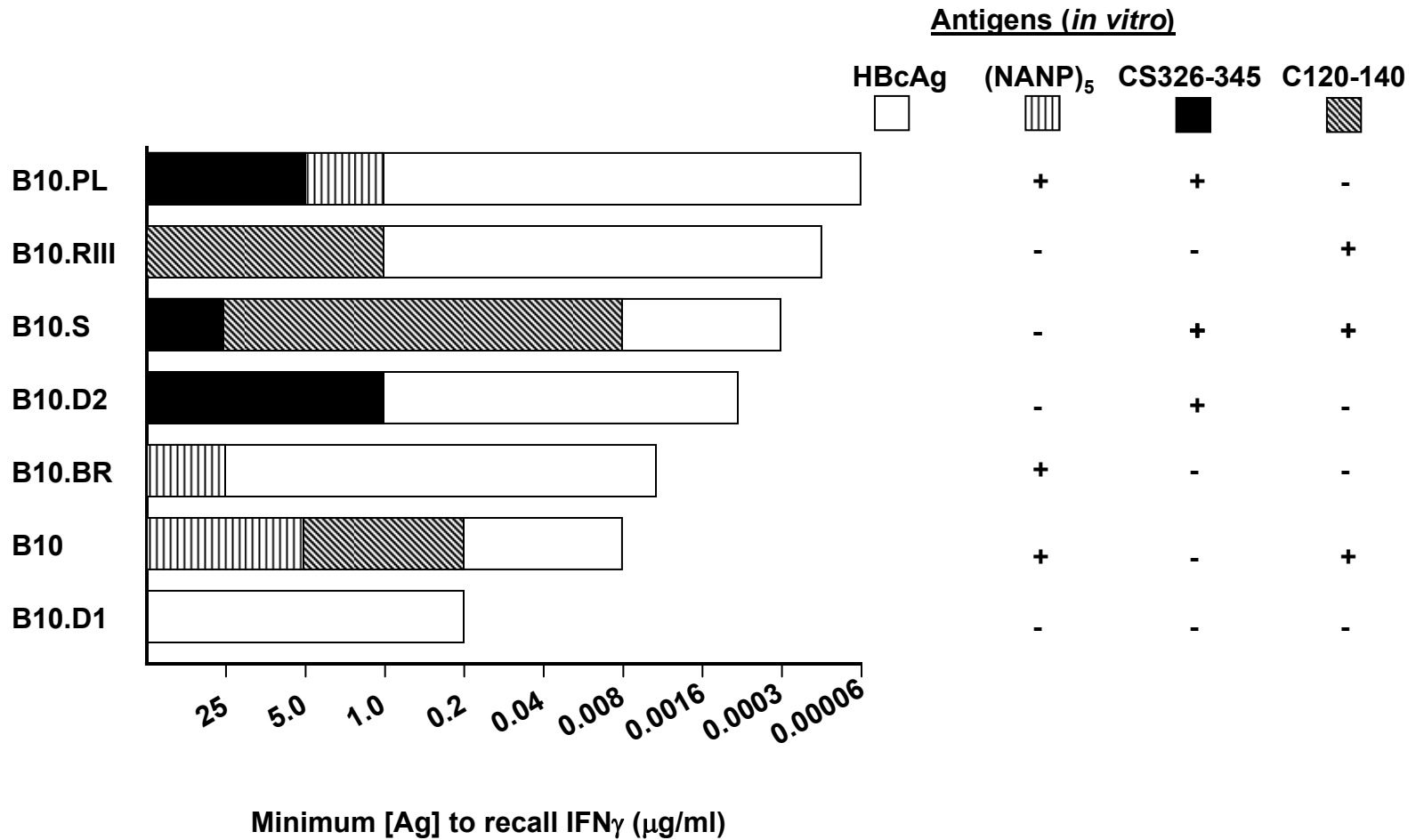
WHc-CS *in vivo* Immunization



Hybrid *P. falciparum*/*P. berghei* Sporozoite *in vivo* Challenge/Protection

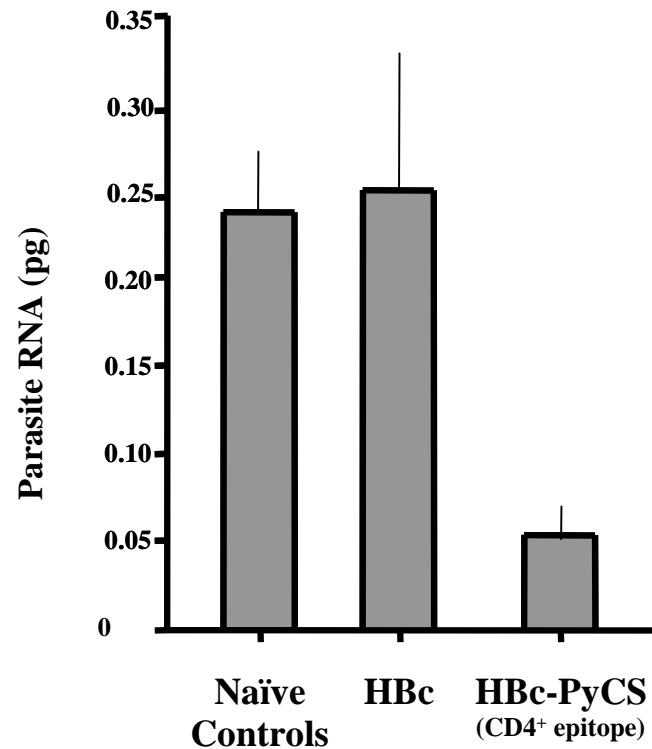


Fine Specificity of T cells Primed by HBc-CS

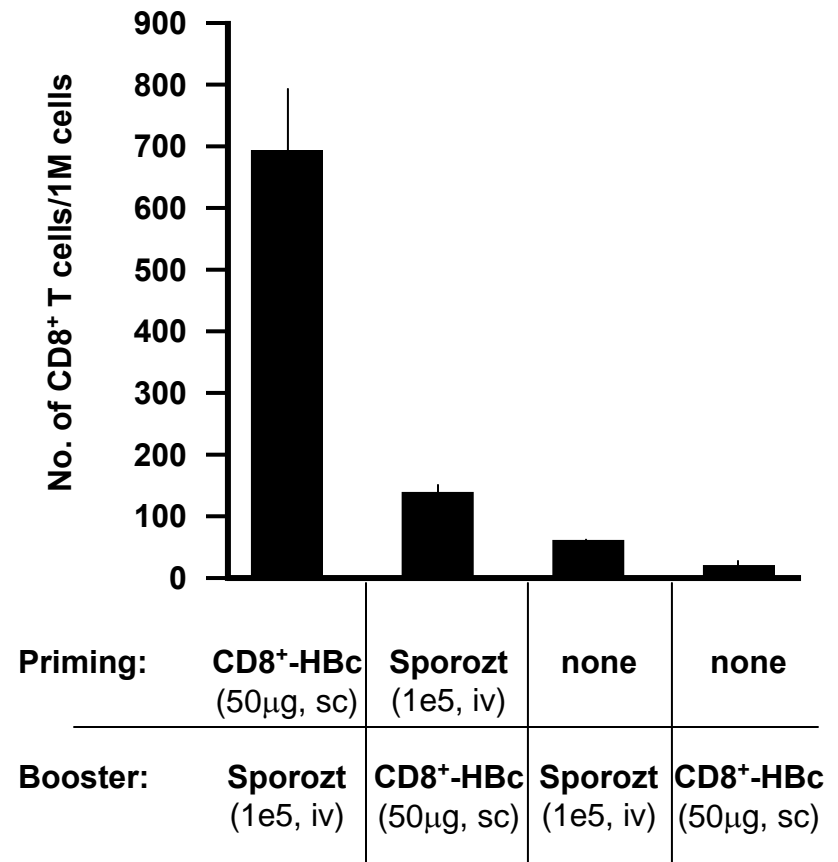
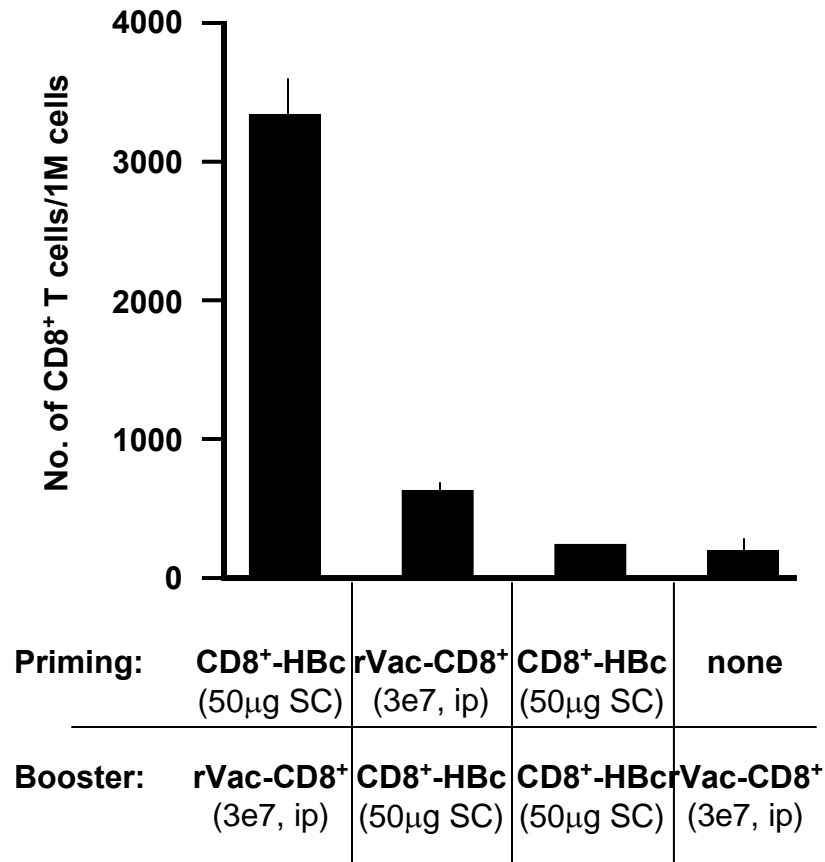


A Core VLP Can Confer Insert Specific CD4⁺ Protection

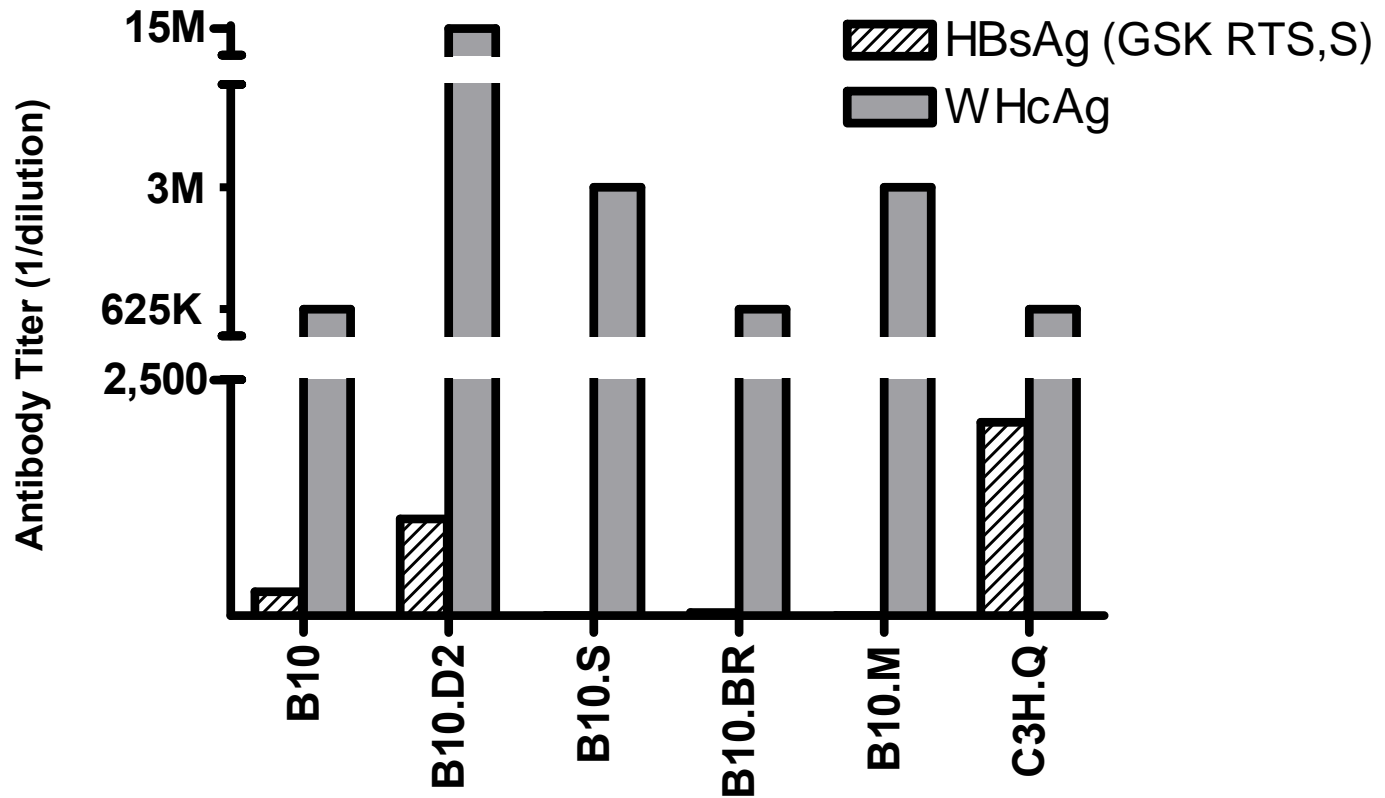
- 1) BALB/c mice were immunized with a chimeric HBc-based VLP in CFA, containing a mouse malaria CD4⁺ T cell site.
- 1) Animals were challenged with 50,000 sporozoites and infection of the liver determined.



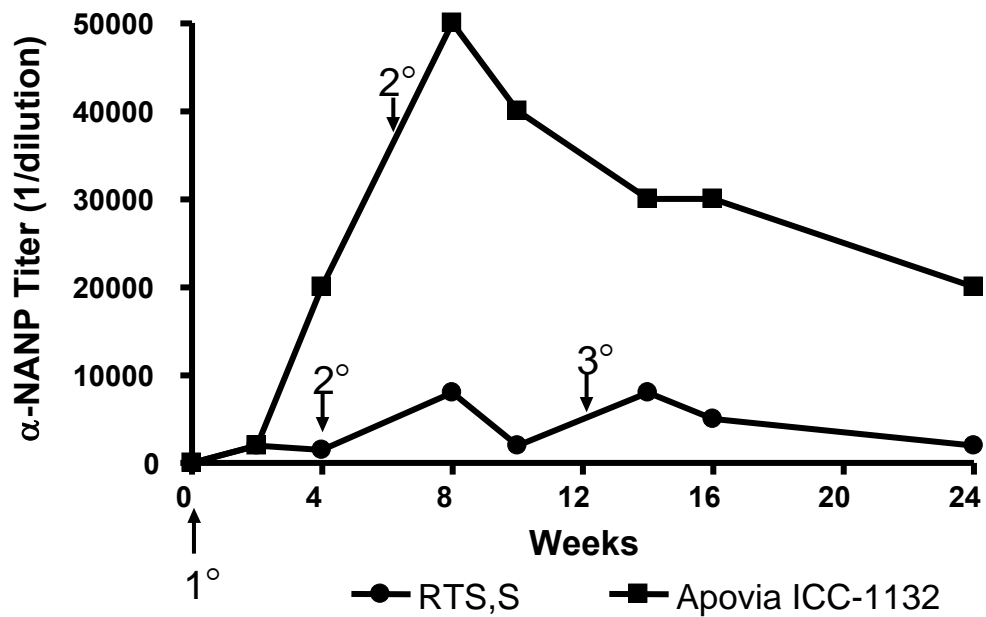
Induction of Malaria-Specific CD8⁺ T cells by Immunization with HBc-CD8⁺ Site VLPs



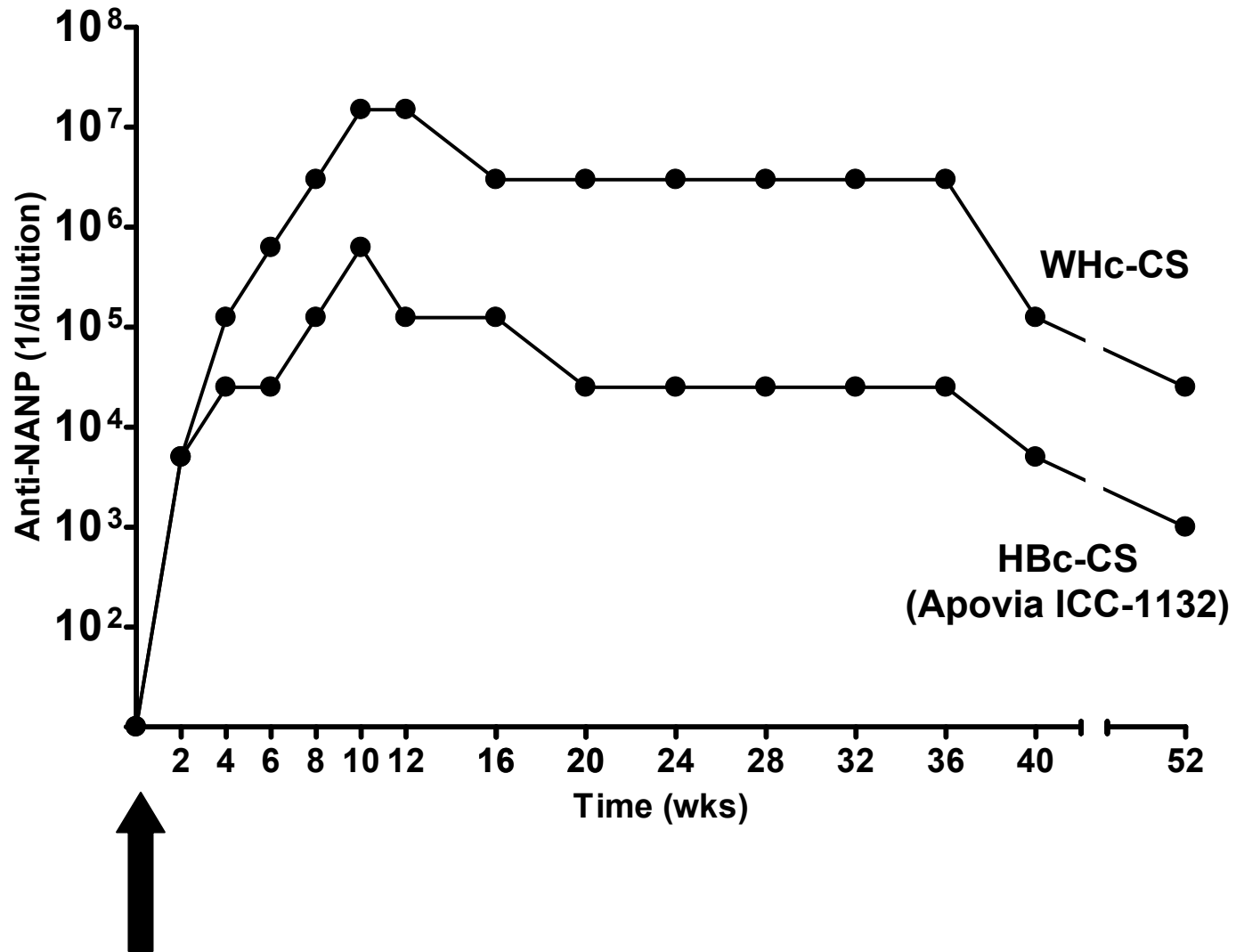
Rationale for the Use of WHc/HBcAg vs HBsAg as a VLP Carrier



Antibody Titers for RTS,S and Apovia ICC-1132 in Rhesus Macaques



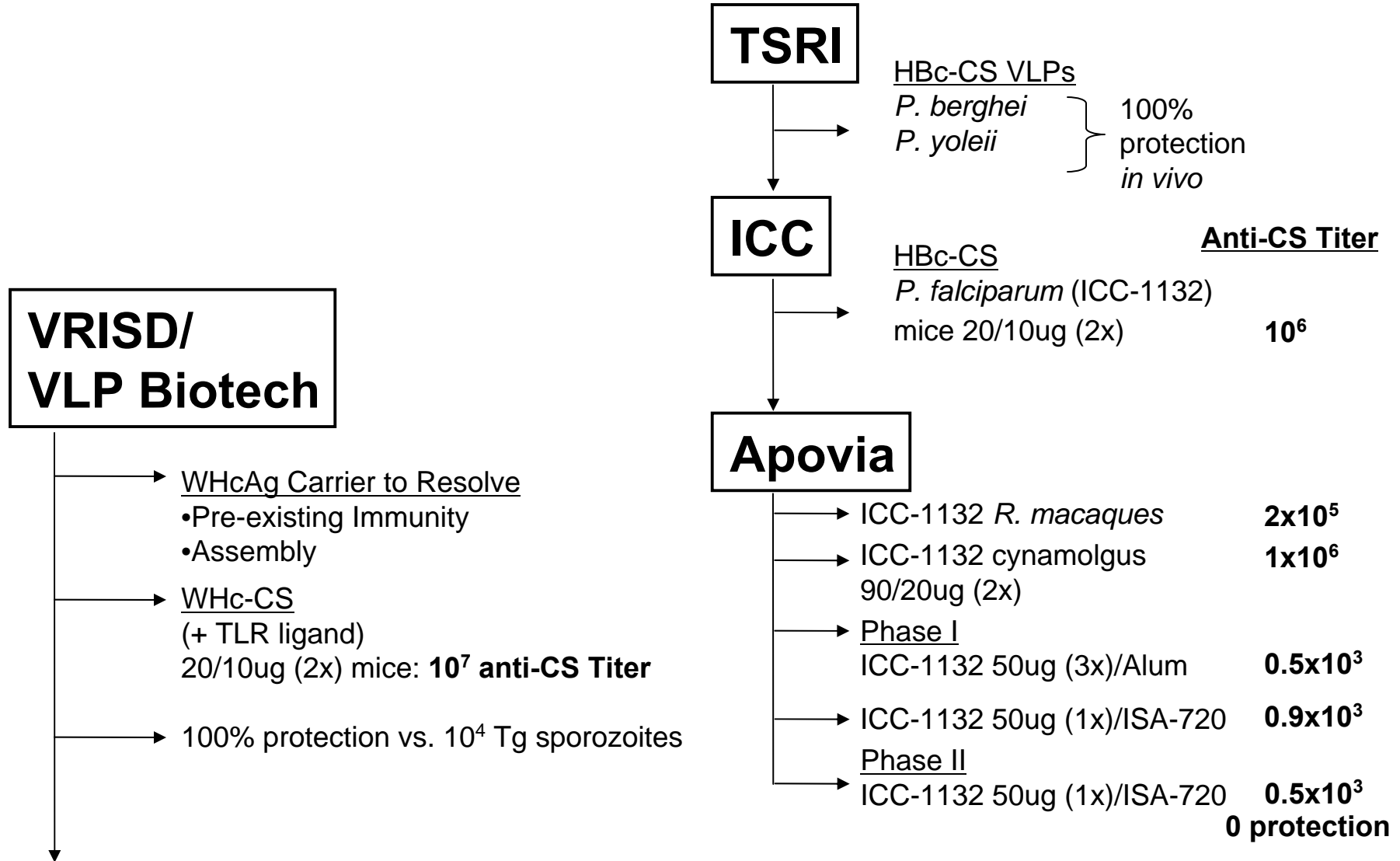
Comparison of WHc vs HBc VLP



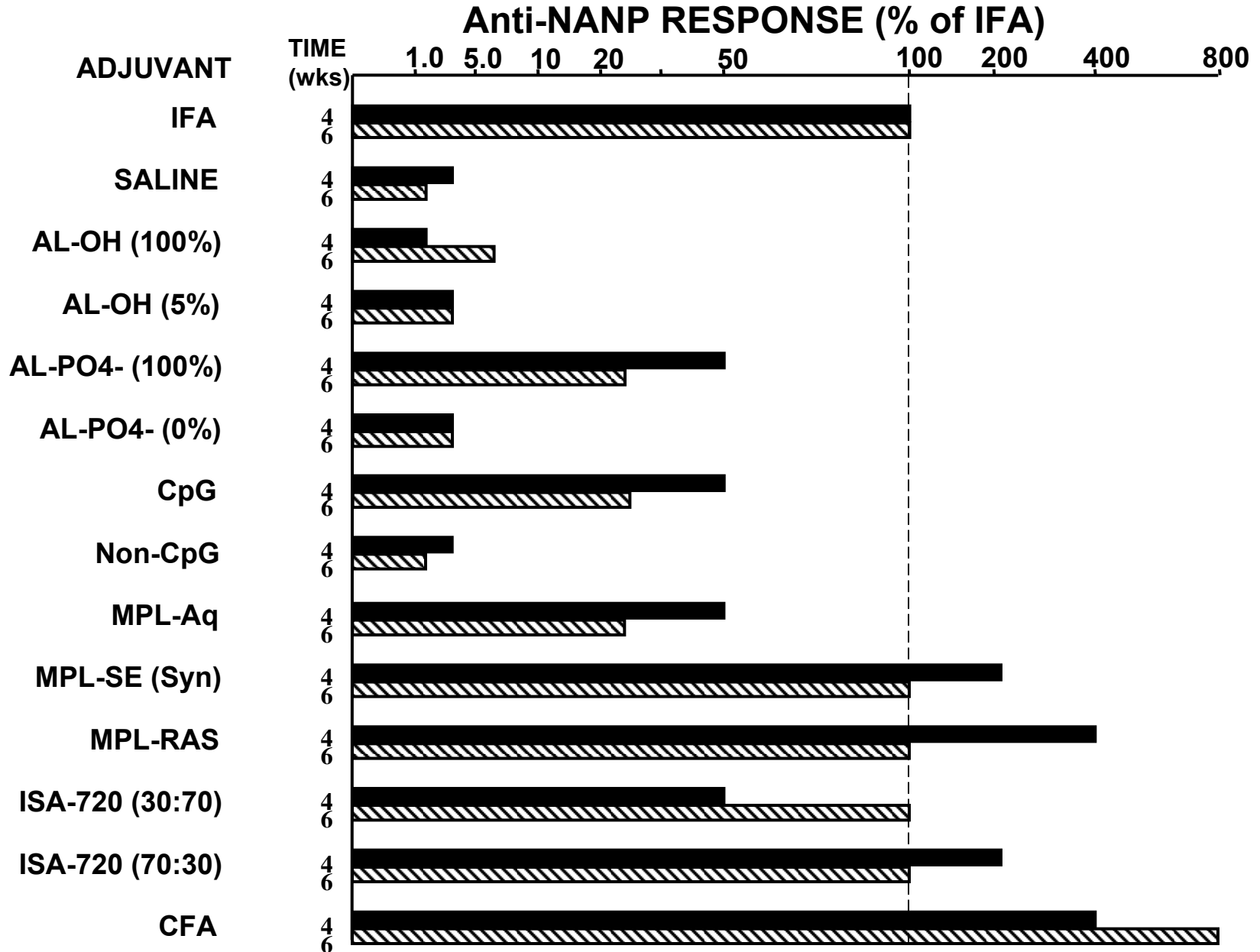
		RTS,S	WHc-CS	Apovia (ICC-1132)
Malaria	B epitopes	CS Repeats: (NANP) ₁₆ only	CS Repeats CS Non-Repeats	CS Repeats
	T epitopes	CS, C-terminus 302-395	CS 318-337 (UTC) CS 339-363 (TH.3R) CS 363-377 (CS.T3)	CS 318-337
Carrier	HBsAg (human pathogen)	WHcAg (full-length) (non-human pathogen)	HBcAg (truncated) (human pathogen)	
	T cell dependent	T cell dependent or independent	T cell dependent or independent	
	100-1000 fold less immunogenic in mice	100-1000 fold more immunogenic in mice	100-1000 fold more immunogenic in mice	
	Soluble HBsAg less immunogenic in humans	N.D.	Soluble HBcAg more immunogenic in humans	
	MHC nonresponder genotypes (mice & human)	No MHC nonresponders identified	No MHC nonresponders identified	
	Pre-existing anti-HBs from HBV infection	No Pre-existing anti-HBs from HBV infection	Pre-existing anti-HBs from HBV infection	
	Immune tolerance in HBV chronics	No immune tolerance in HBV chronics	Immune tolerance in HBV chronics	
	N.D.	Delete carrier B cell sites	N.D.	
	Requires co-expression of HBsAg for HBsAg-CS assembly	Self assembly of WHc-CS	Self assembly of HBc-CS	
	Cannot express in bacteria	Bacterial expression	Bacterial expression	
	N.D.	100% protection vs 10k Tg sporozoites	N.D.	

	RTS,S	WHc-CS	Apovia (ICC-1132)
Endogenous Molecular Adjuvants	None	ssRNA – TLR Ligands	None
Stability to Fixation	Lyophilization	Lyophilization	?
Cold Chain	Not Required	Not Required	Required
Formulation/ Adjuvants	Absolute requirement for immunostimulatory adjuvant (AS02A, MPL/QS21/oil)	to be determined	Montanide 720, Alum
Phase II Clinical Results	1-3 dose, 0 protection	N.D.	1 dose, 0 protection
	3 dose +AS02A, 30-50%	N.D.	N.D.

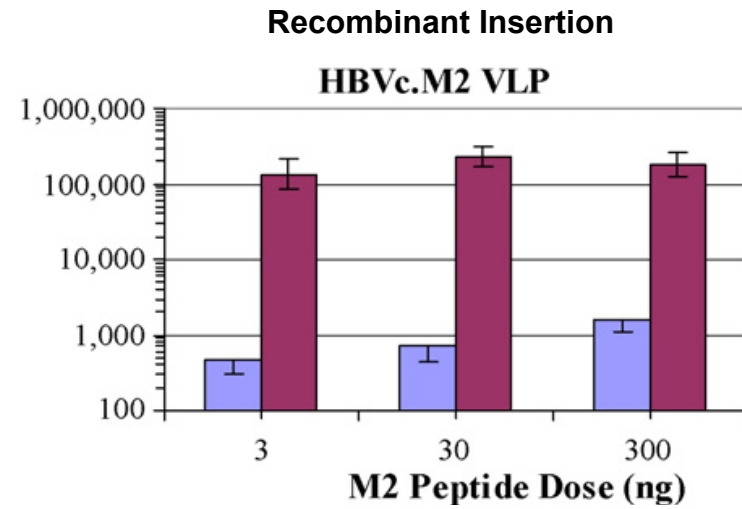
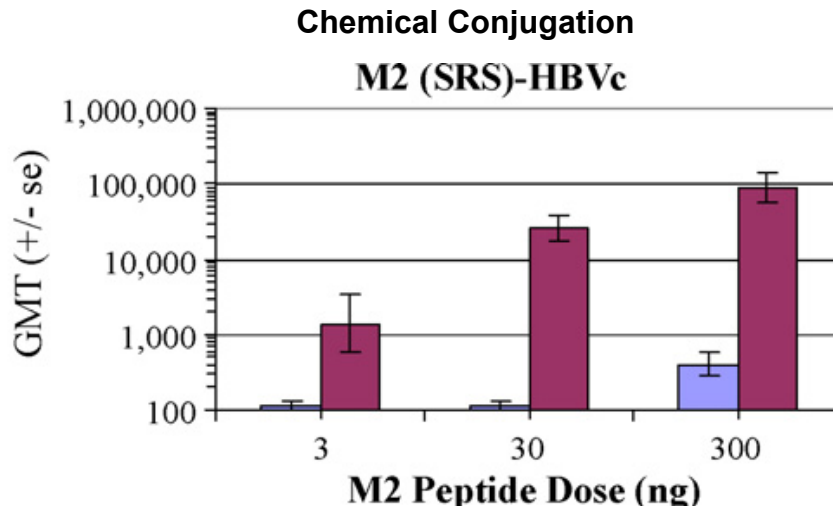
Evolution of the Hepadnaviral Core-Based Malaria VLP Vaccine



Influence of Adjuvant on Hybrid WHc VLP Immunogenicity



Chemical Conjugation vs. Recombinant Insertion



Summary (1)

- **WHcAg-VLP is a potent and flexible vaccine carrier**
 - **Inexpensive production in bacteria**
 - **Fully recombinant technology (not limited to conjugation)**
 - **Display of 240 epitopes per VLP**
 - **Compatible with lyophilization**
- **Derived from a non-human pathogen**
- **WHcAg combinatorial tools enable efficient assembly of chimeric VLPs with most inserts**

Summary (2)

- **The WHcAg-VLP can encapsidate TLR ligands**
 - **Avoids potential toxicity from systemic delivery of adjuvant**
 - **Direct delivery of immune stimulant to specific APC**
 - **Co-ligation of BCR and TLR**
 - **Ligand protected from nucleases and proteases**
- **Deletion of carrier B cell epitopes**
 - **Higher anti-insert immunogenicity/protection**
 - **Improves use for multiple vaccines**
- **HBc/WHc-CS VLPs demonstrated protection against:**
 - *P. falciparum*
 - *P. yoelii*
 - *P. berghei*

Collaborators

VRISD

Immunology:

Dave Milich
Byung Lee
Joyce Jones
Cory Peters

Molecular Biology:

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Bryan Darsow

VCU

Protein Chemistry:

Darrell Peterson

JHSPH

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