Epstein-Barr Virus Vaccines

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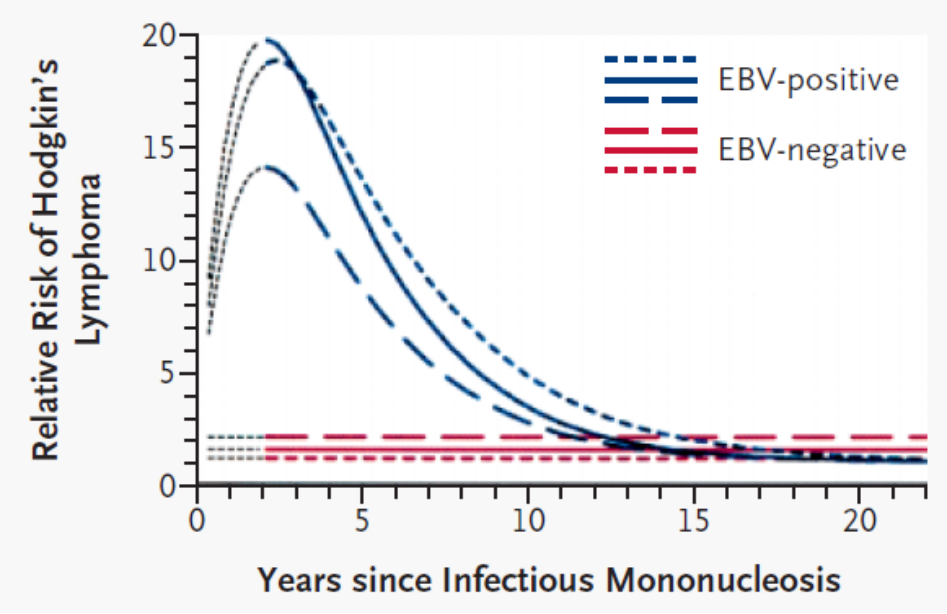
Primary EBV Infection

- 95% of adults are infected with EBV
- Most primary EBV infections in childhood are asymptomatic
- 75% of college students with primary EBV infection will develop infectious mononucleosis
- 1:1,000 persons with EBV mononucleosis will develop Hodgkin lymphom



Hjalgrim et al
NEJM 2003

EBV-Associated Malignancies: Criteria

- EBV is in every tumor cell
- EBV is clonal (or oligoclonal), indicating malignancy originated in a virus-infected cell
- At least one EBV gene is expressed
- For epithelial cell cancers, EBV is in dysplastic lesions, indicating virus infection is present during oncogenesis

The Need for an EBV Vaccine: Malignancies in Non-Immunocompromised Persons

Cancer	Annual No. Cases Associated with EBV
Gastric carcinoma	84,050 worldwide
Nasopharyngeal carcinoma	78,100 worldwide
Hodgkin lymphoma	28,600 worldwide
Burkitt lymphoma	6,600 worldwide

Others:

T cell lymphoma (peripheral, AILT)

Diffuse large B cell lymphoma of elderly

Extranodal NK/T cell lymphoma

Cohen et al.
Sci Trans Med 2011

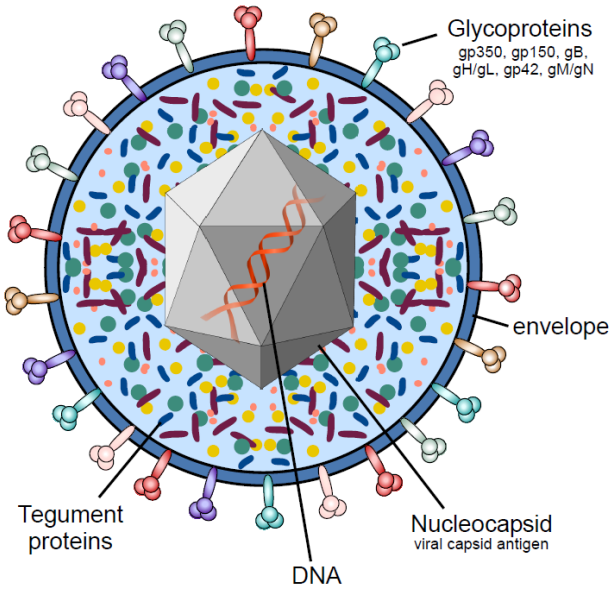
The Need for an EBV Vaccine: Malignancies in Immunocompromised Persons

Post-transplant lymphoproliferative disease: most common cancer after skin cancer in solid organ transplant recipients

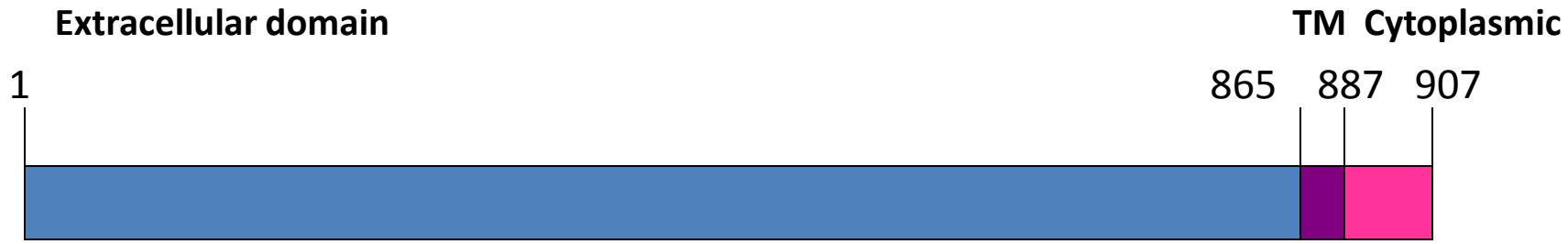
Patients with HIV	Frequency of EBV
Hodgkin lymphoma	>95%
Burkitt lymphoma	30-70%
DLBCL: immunoblastic	>90%
centroblast	30%
Primary CNS lymphoma	>95%
Primary effusion lymphoma	>90%
Smooth muscle tumors	>95%
Plasmablastic lymphoma	50-80%

Vaccine Candidates: EBV Glycoproteins

Glycoprotein	Receptor	Function
gp350	CR2 (CD21)	Attachment to cells
gB	Unknown	Fusion
gp42	MHC class II	Fusion with B cells
gH/gL	αvβ6, αvβ8 integrin	Fusion with epithelial & B cells
BMRF2	Integrins	Attachment to epithelial cells



EBV gp350



- Most abundant viral protein in infected cell plasma membrane and virion envelope
- Binds to CR2 (CD21) on B cells, results in adsorption of virus to cells followed by endocytosis into cells
- Little sequence variation in gp350 among isolates throughout the world
- gp350 is the principal target of neutralizing antibodies to EBV
- gp350 is a target for antibody-dependent cellular cytotoxicity
- gp350 is a target for cytotoxic T cells

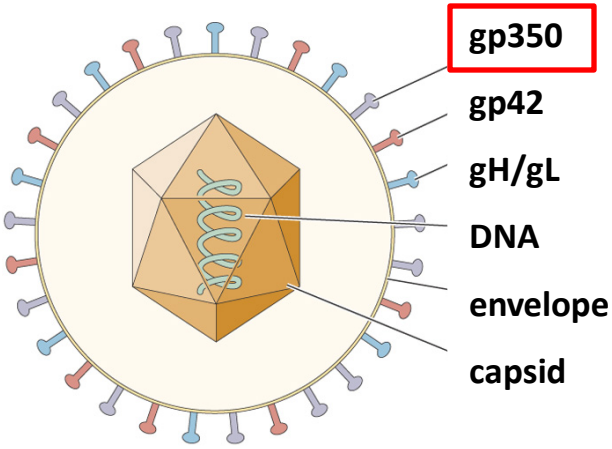
Protection of cottontop tamarins against Epstein–Barr virus-induced malignant lymphoma by a prototype subunit vaccine

M. A. Epstein, A. J. Morgan, S. Finerty,
B. J. Randle & J. K. Kirkwood

Department of Pathology, University of Bristol Medical School,
University Walk, Bristol BS8 ITD, UK

NATURE VOL. 318 21 NOVEMBER 1985

"Here we report that isolated cell membranes expressing MA or purified MA glycoprotein [gp350 in liposomes], have been used to vaccinate cottontop tamarins, and that animals receiving either preparation were protected against the effects of a 100% tumor-inducing challenge dose of EB virus."



Also effective: purified/recombinant gp350 in ISCOMs, muramyl dipeptide, alum
adenovirus-gp350 or vaccinia virus-gp350

Recombinant gp350 Vaccine for Infectious Mononucleosis: A Phase 2, Randomized, Double-Blind, Placebo-Controlled Trial to Evaluate the Safety, Immunogenicity, and Efficacy of an Epstein-Barr Virus Vaccine in Healthy Young Adults

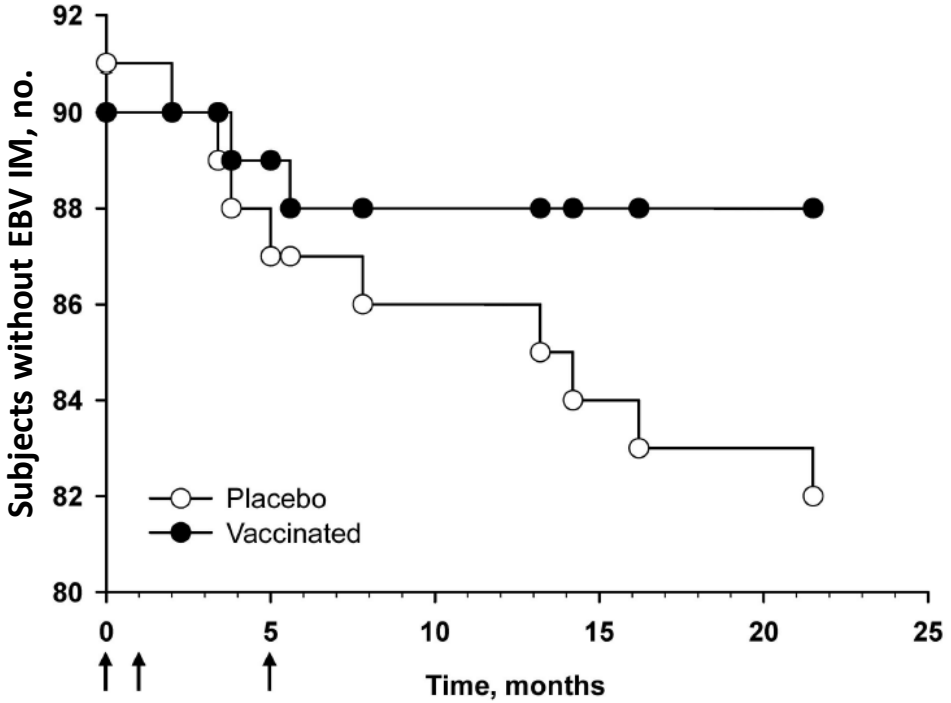
The Journal of Infectious Diseases 2007; 196:1749–53

Etienne M. Sokal,[1] Karel Hoppenbrouwers,[3] Corinne Vandermeulen,[3] Michel Moutschen,[4] Philippe Léonard,[4] Andre Moreels,[2] Michèle Haumont,[5] Alex Bollen,[5] Françoise Smets,[1] and Martine Denis[6]

[1]Université Catholique de Louvain, Cliniques Universitaires St-Luc, and [2]Vrije Universiteit Brussel, Campus Etterbeek, Brussels, [3]Department of Youth Health Care, Katholieke Universiteit Leuven, Leuven, [4]Laboratory of Pathology, University of Liège, Liège, [5]Henogen, Gosselies, and [6]GlaxoSmithKline Biologicals, Rixensart, Belgium

16-25 yo Belgians vaccinated:
- 90 received vaccine
- 91 received placebo

Vaccine given i.m. in alum/MPL adjuvant at 0,1, 5 months



gp350 vaccine reduced infectious mononucleosis by 78%, but did not prevent EBV Infection

Phase I Trial of a CD8$^+$ T-Cell Peptide Epitope-Based Vaccine for Infectious Mononucleosis$^{\nabla}$

Suzanne L. Elliott,[1] Andreas Suhrbier,[1]* John J. Miles,[1] Greg Lawrence,[1] Stephanie J. Pye,[1] Thuy T. Le,[1] Andrew Rosenstengel,[1] Tam Nguyen,[1] Anthony Allworth,[2] Scott R. Burrows,[1] John Cox,[3] David Pye,[3] Denis J. Moss,[1] and Mandvi Bharadwaj[1]*

Australian Centre for Vaccine Development, Queensland Institute of Medical Research, Brisbane, Australia[1]; Infectious Disease Unit, Royal Brisbane Hospital, Brisbane, Australia[2]; and CSL Limited, Melbourne, Australia[3]

JOURNAL OF VIROLOGY, Feb. 2008, p. 1448–1457

HLA B*0801 EBNA-3A peptide and tetanus toxoid:

1 of 2 placebo recipients developed IM

4 of 4 vaccinees who became infected with EBV did not develop IM

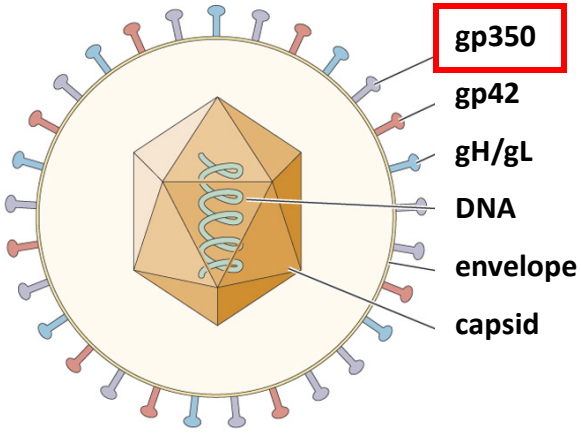
Vaccine	Vaccine recipient	EBV seroconversion (wk of test)	IM or asymptomatic
Peptide 5 μg	#01	No (412)	
	#02	Yes (628)	Asymptomatic
	#04	Yes (104)	Asymptomatic[d]
	#05	No (542)	
	#06	No (523)	
	#07[a]	No (520)	
	#08[a]	Yes (104)	Asymptomatic[d]
	#09[b]	Yes (26)	Asymptomatic[d]
50 μg	#13	No (421)	
	#14[c]	Yes (8)	?[d,e]
Placebo	#03	No (585)	
	#10	No (494)	
	#11[a]	Yes (438)	Asymptomatic
	#12	Yes (392)	IM, treating doctor notified

Rhesus Lymphocryptovirus (Rhesus EBV) to Compare EBV Vaccines

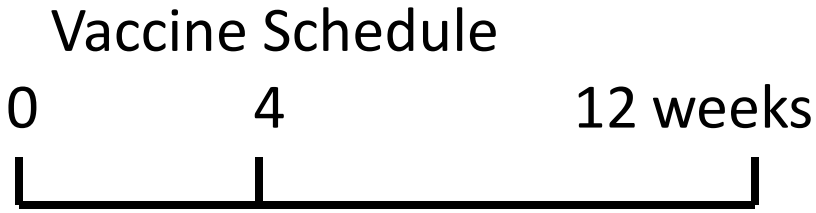
- Naturally endemic in rhesus monkeys and reproduces most, if not all, of the features of EBV in these animals *(Moghaddam et al. 1997)*
- Animals shed virus from oropharynx, and are latently infected
- Every human EBV gene has an ortholog in rhesus LCV and vice versa
- Functions of EBV and rhesus LCV proteins conserved

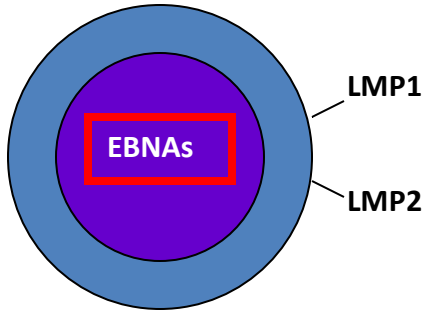
Rhesus Lymphocryptovirus (Rhesus EBV) Vaccine Study

- Soluble rhesus LCV gp350 (50ug) in alum/MPL
- Attenuated VEE virus-like replicon particles (VEE VRP) expressing rhesus EBV gp350 10^8 ID i.m.
- VEE VRP expressing rhesus LCV gp350, EBNA-3A, EBNA-3B 10^8 ID i.m.



gp350 is major target of neutralizing Ab induces some CD4 T cells

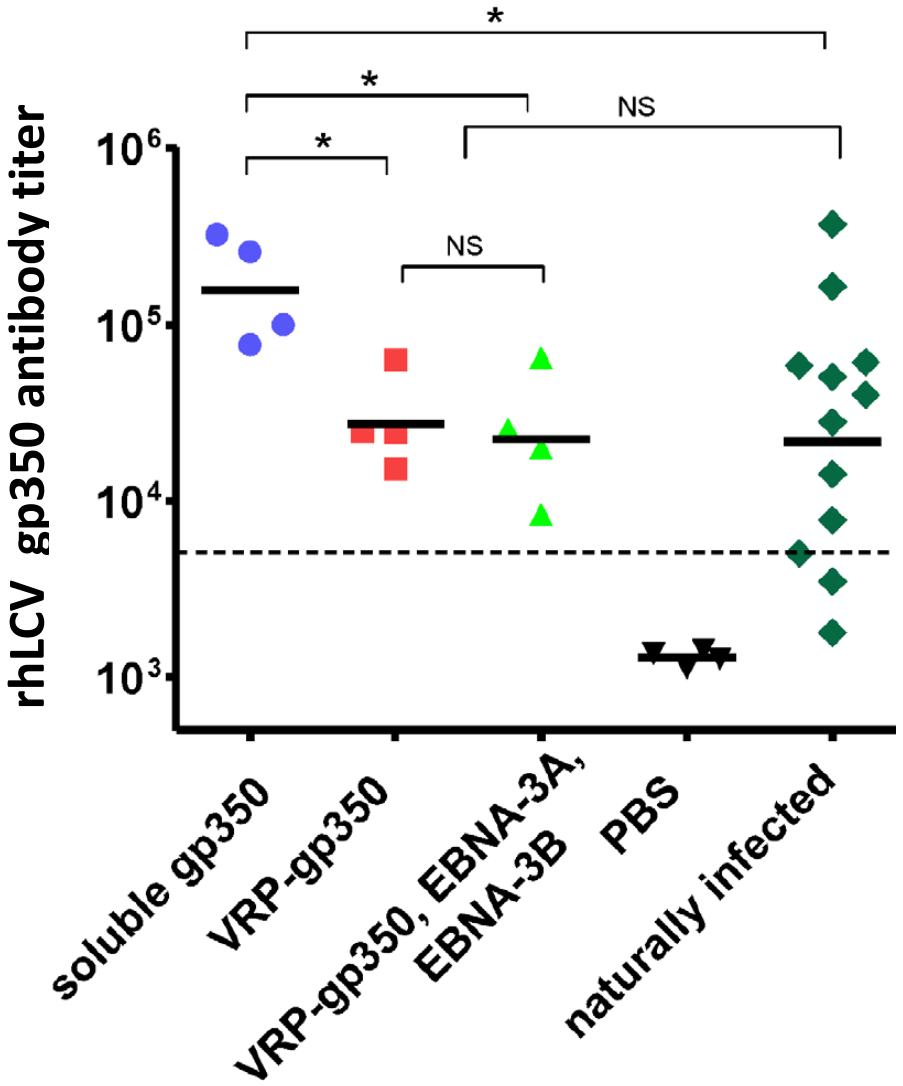




EBV latently-infected B cell

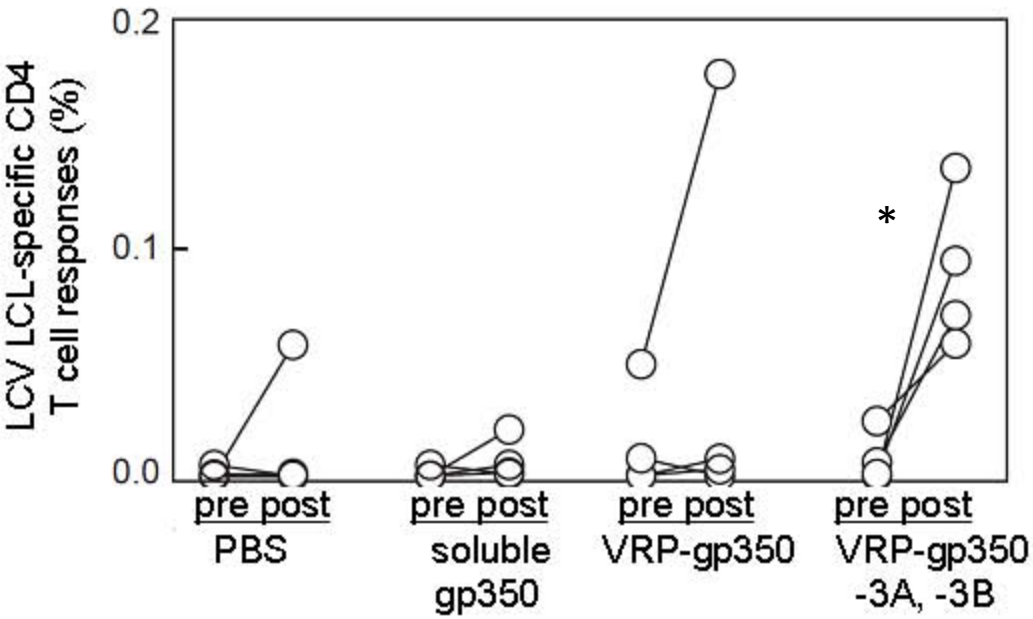
EBNA-3s are major target of CD8 CTLs

Soluble Rhesus LCV gp350 Induces the Highest Antibody Level After Vaccination

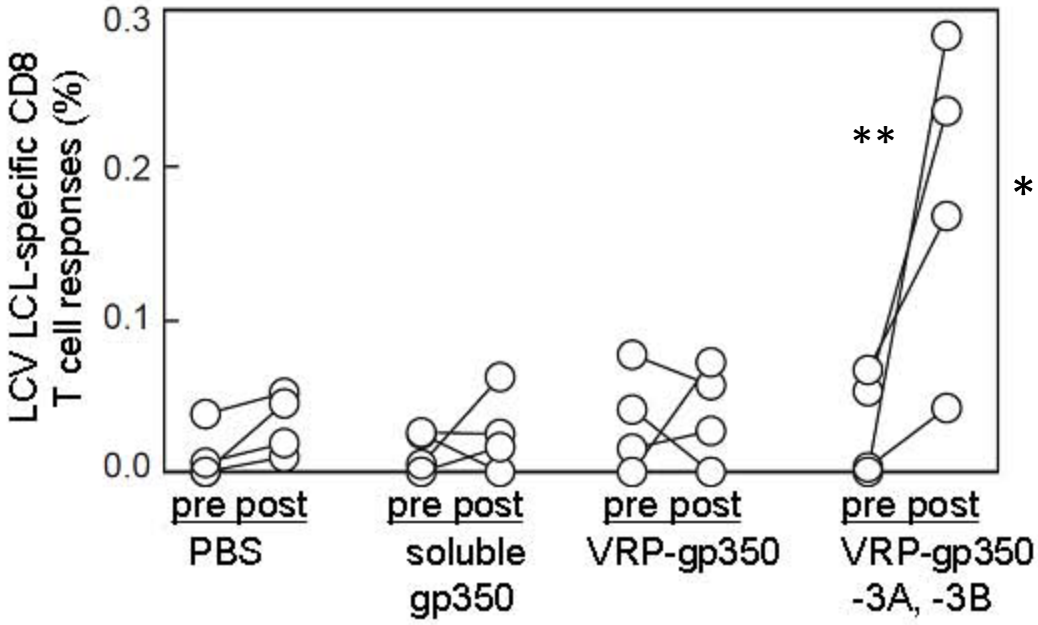


Sashihara et al.
PLOS Pathogens 2011

Only the Combination of VRP-gp350, EBNA-3A, & -3B Induces Rhesus LCV-Specific CD4 and CD8 T Cell Responses



*p=0.032



**p=0.065

Soluble gp350 Protects Best Against Rhesus EBV Challenge

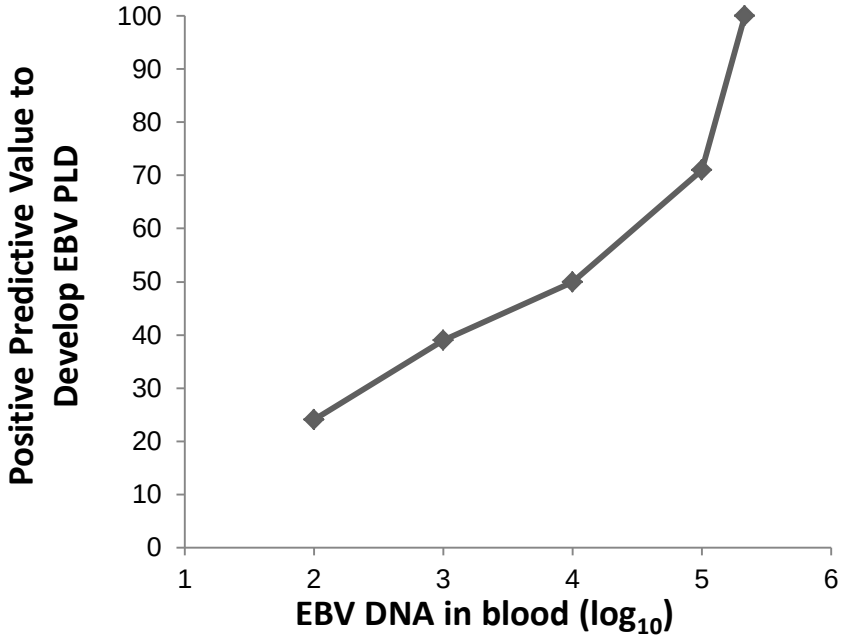
Vaccine	Seroconvert	EBV DNA	EBV RNA
PBS	100%	75%	100%
Soluble gp350	50%	50%	50%
VRP-gp350	100%	100%	100%
VRP-combo	75%	75%	50%

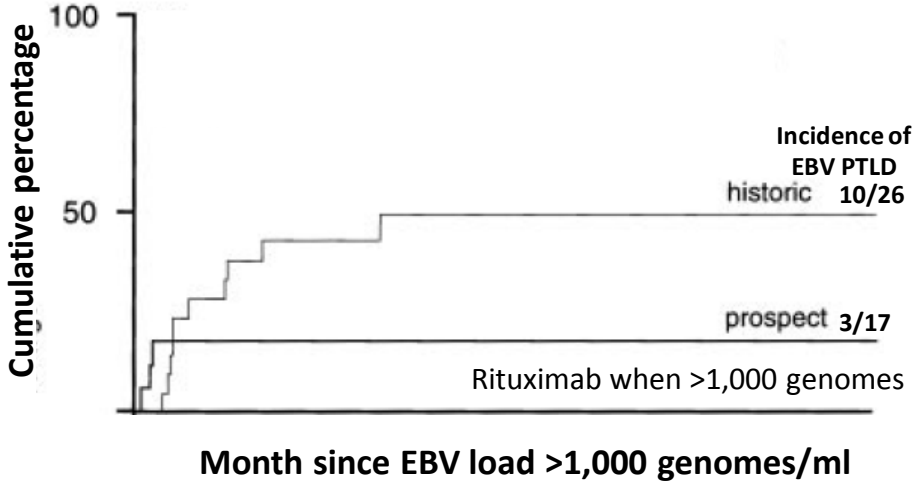
EBV PTLD- EBV DNA Viral Load

EBV viral load in blood partially predictive of disease in transplant recipients

Rituximab (anti-CD20 MAb), given when viral load is increasing, before the onset of EBV PTLD, usually results in an undetectable viral load and may reduce the disease

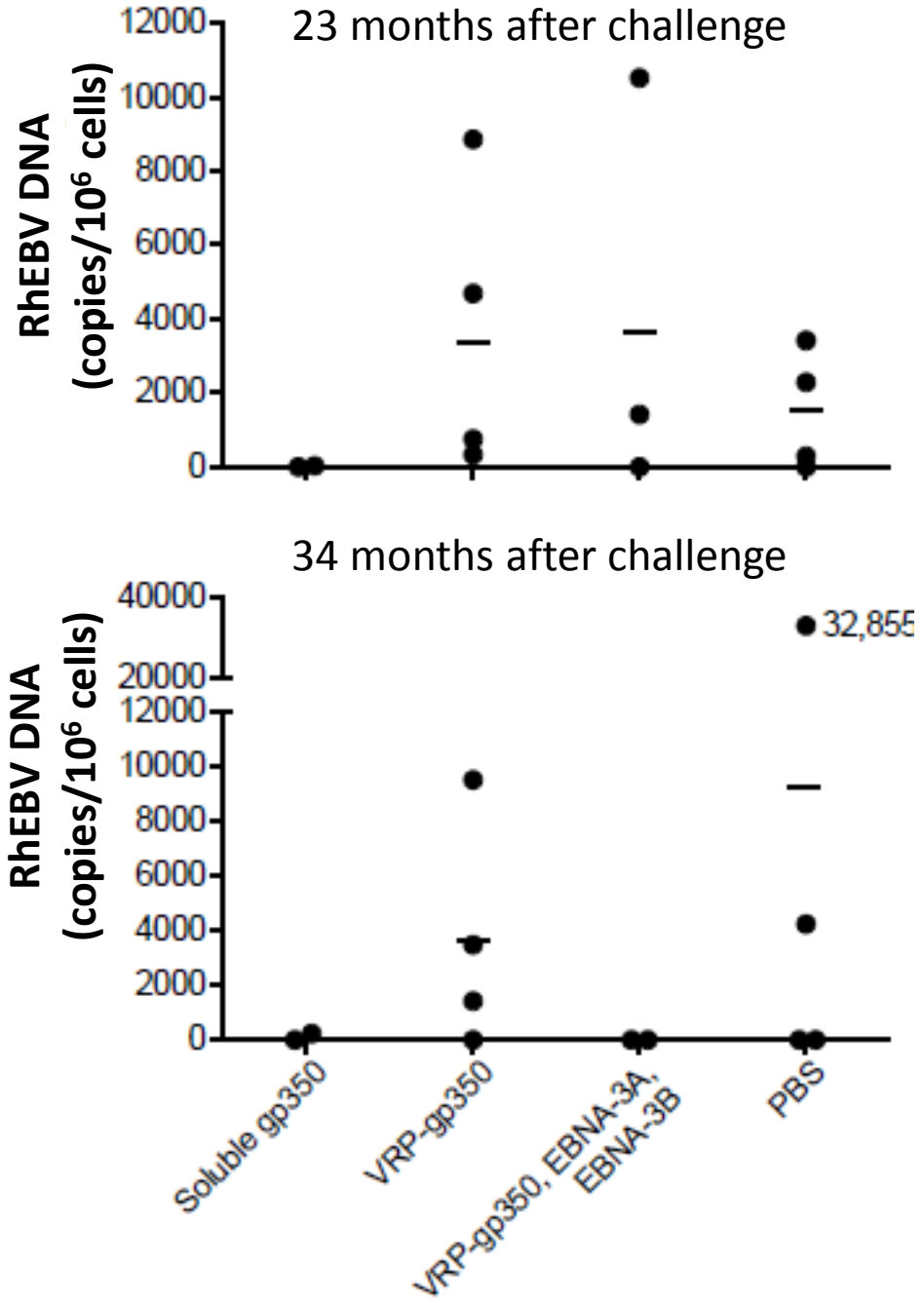
Therefore a vaccine that doesn't prevent infection, but reduces EBV load set point might reduce the rate of EBV PTLD





Van Esser Blood 2001,2002

Soluble gp350 Is Best to Reduce Rhesus EBV DNA in Blood Years Later in Animals that Become Infected After Challenge

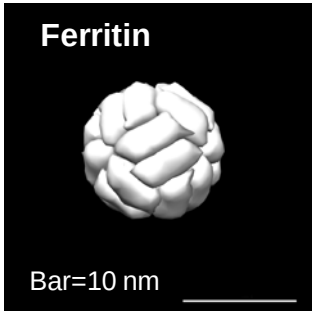


Since level of EBV DNA in blood is a risk factor for EBV lymphoma after transplant, soluble gp350 might reduce the risk of certain EBV lymphomas

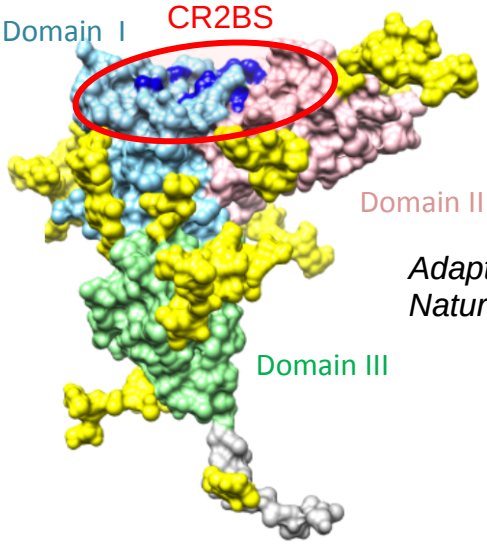
Can We Improve Upon Soluble gp350: Self-Assembling Nanoparticle Based Vaccines

- Multivalent, symmetrical repetitive array of antigen to improve immunogenicity
- Self-assembles into 24-mer
- Proof of Concept:

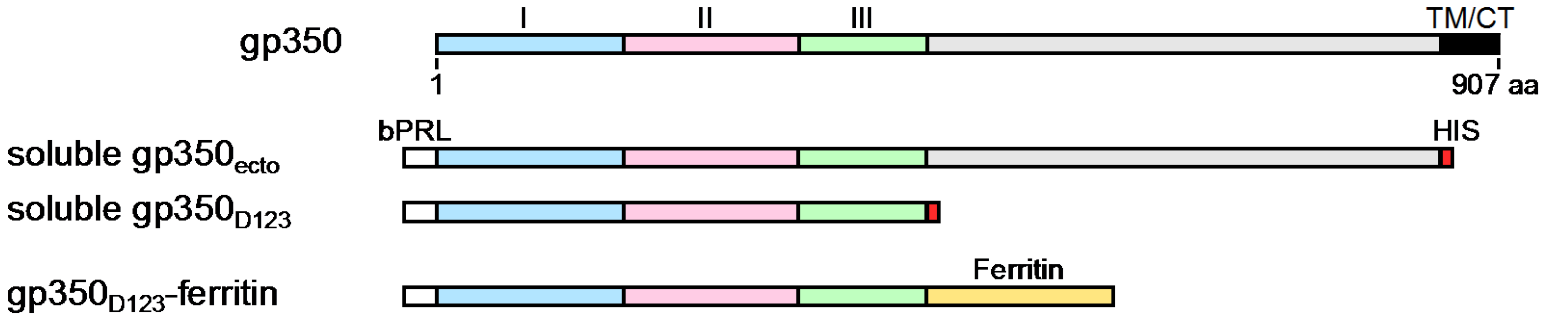
  Ferritin-influenza HA induced high titer, broadly neutralizing antibodies in mice (*Kanekiyo et al Nature 2013*)



Molecular Design of EBV gp350-Nanoparticles



Adapted from Szakonyi et al.
Nature Struct Mol Biol 2006

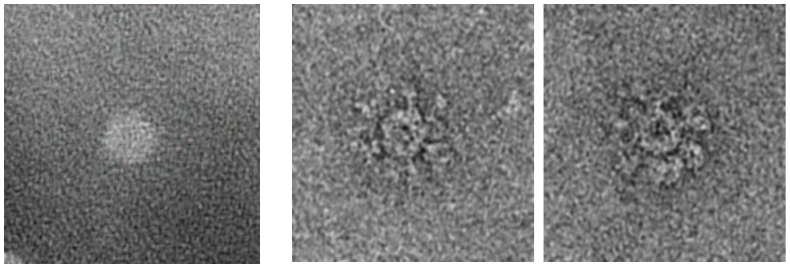


Characterization of EBV gp350-Nanoparticles

Transmission electron microscopy

ferritin

$gp350_{D123}$-ferritin



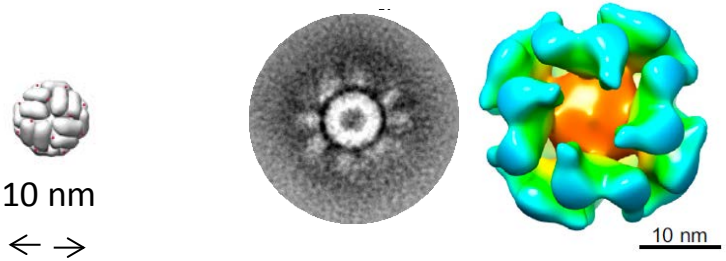
10 nm

50 nm

50 nm

ferritin

$gp350_{D123}$-ferritin



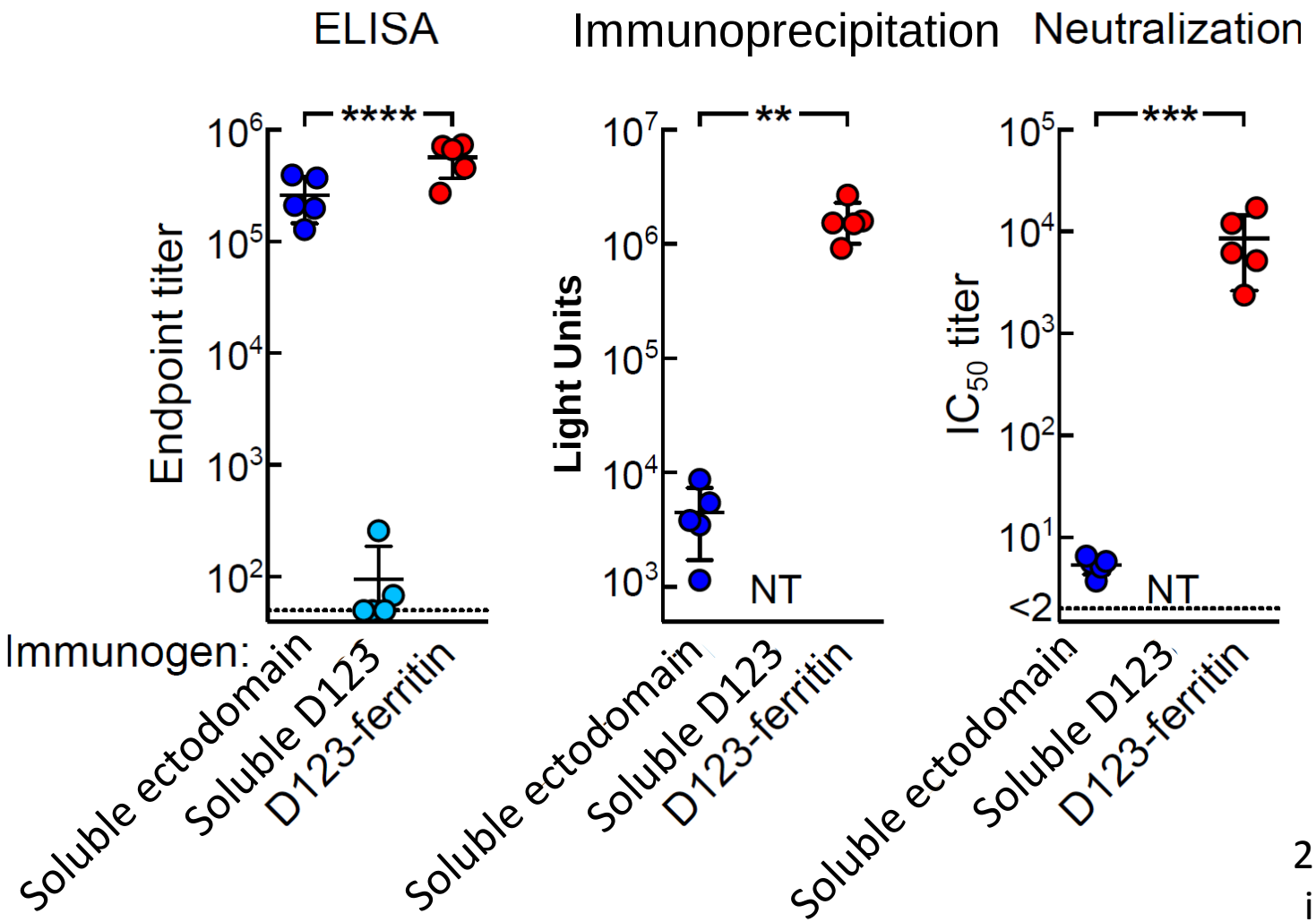
10 nm

EM
2D averaged

CryoEM
reconstruction

Kanekiyo et al Cell 2015

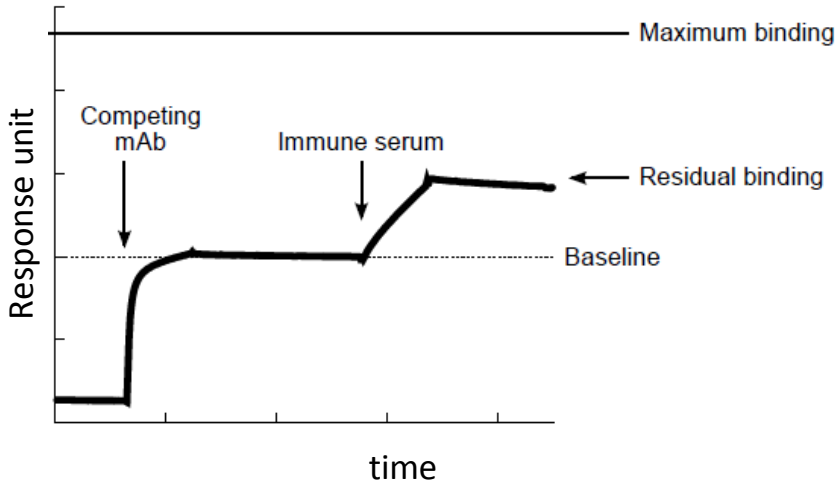
Ferritin-gp350 Induces Higher B Cell Neutralizing Titers than Soluble gp350 in Mice

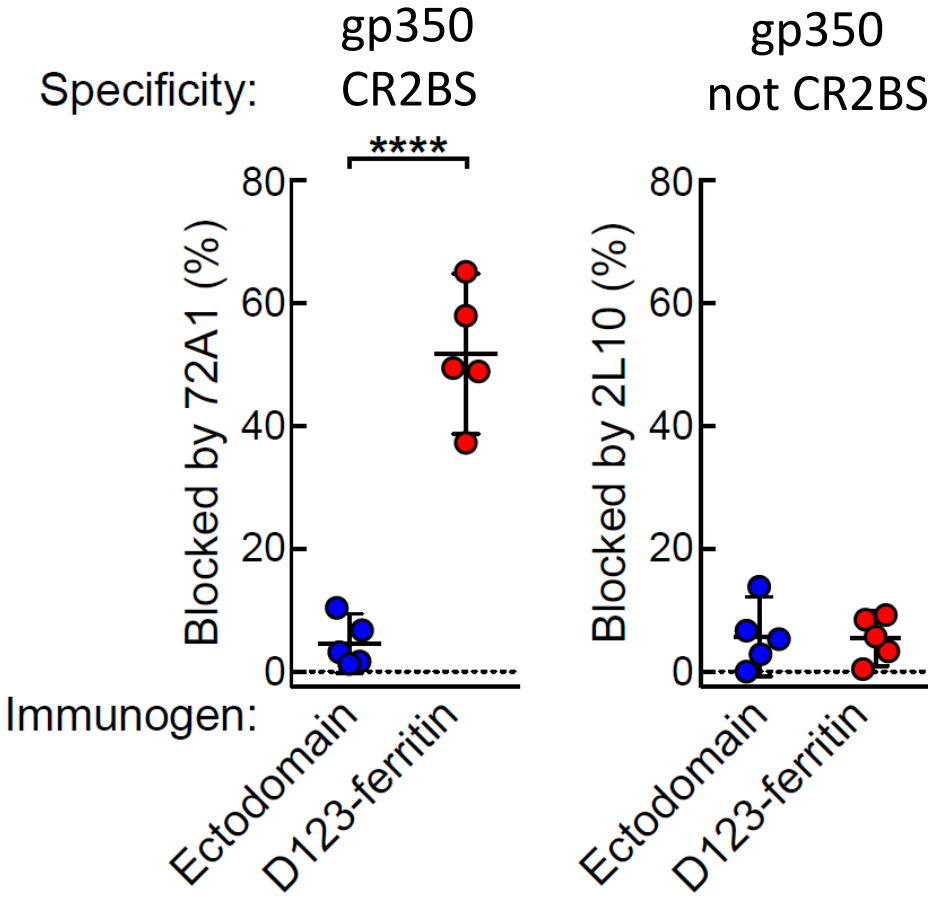


2 doses in Ribi adjuvant

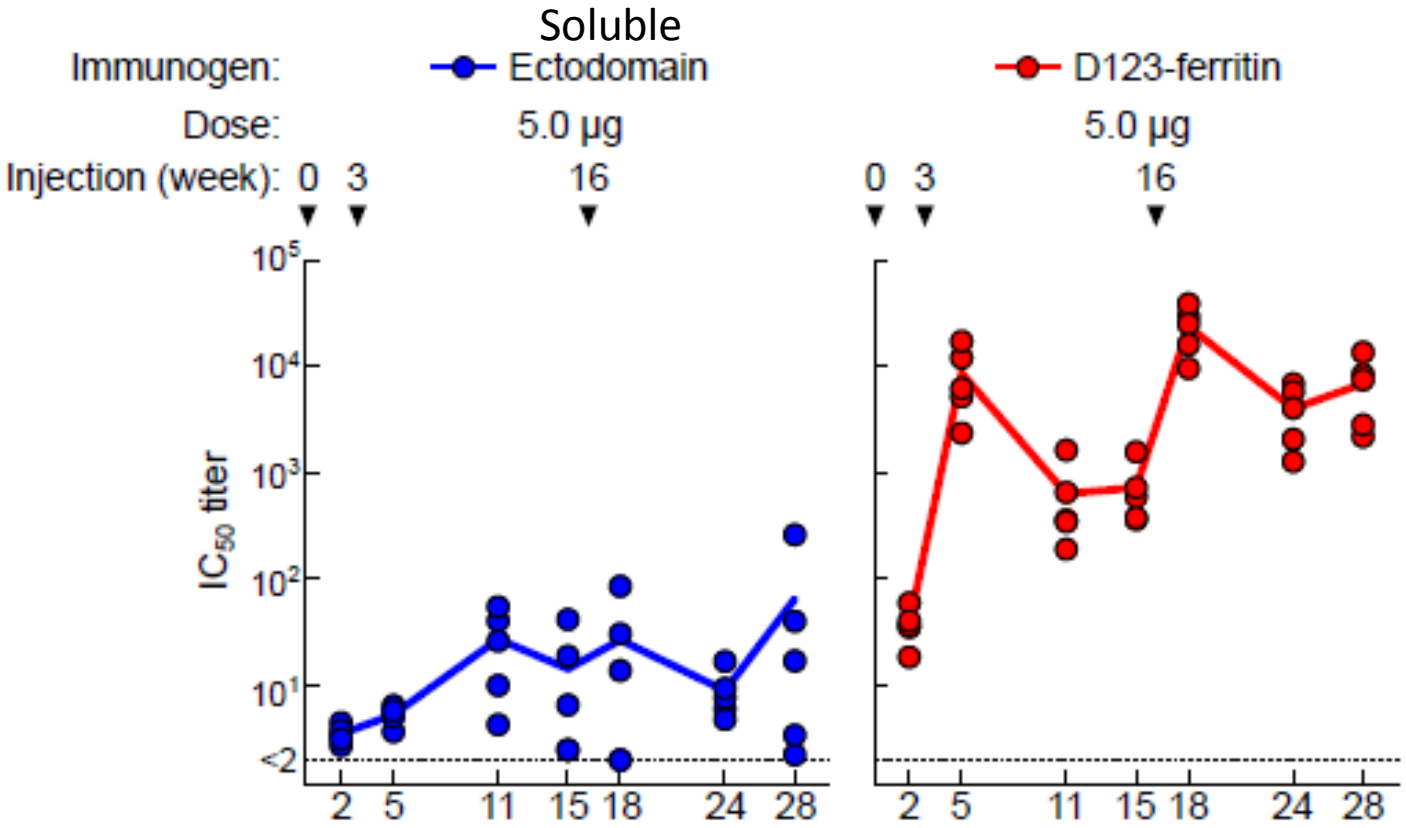
Ferritin-gp350 Induces Higher Levels of Antibody to the CR2 Binding Site (CR2BS) than Soluble gp350 in Mice

Surface Plasmon Resonance
with gp350 on sensor chip

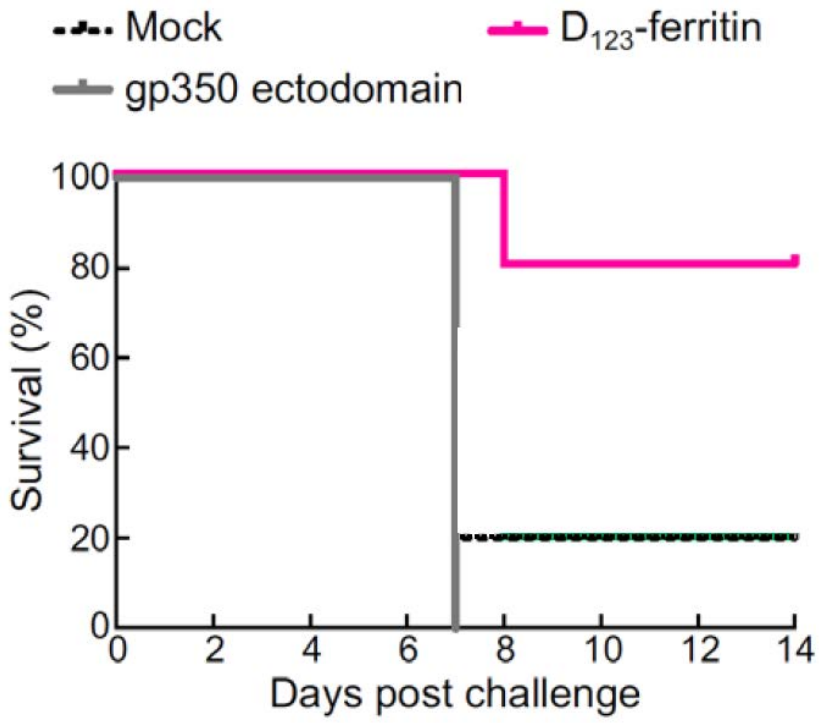




Ferritin-gp350 Neutralizing Antibody Persists for Longer than Soluble gp350 in Mice

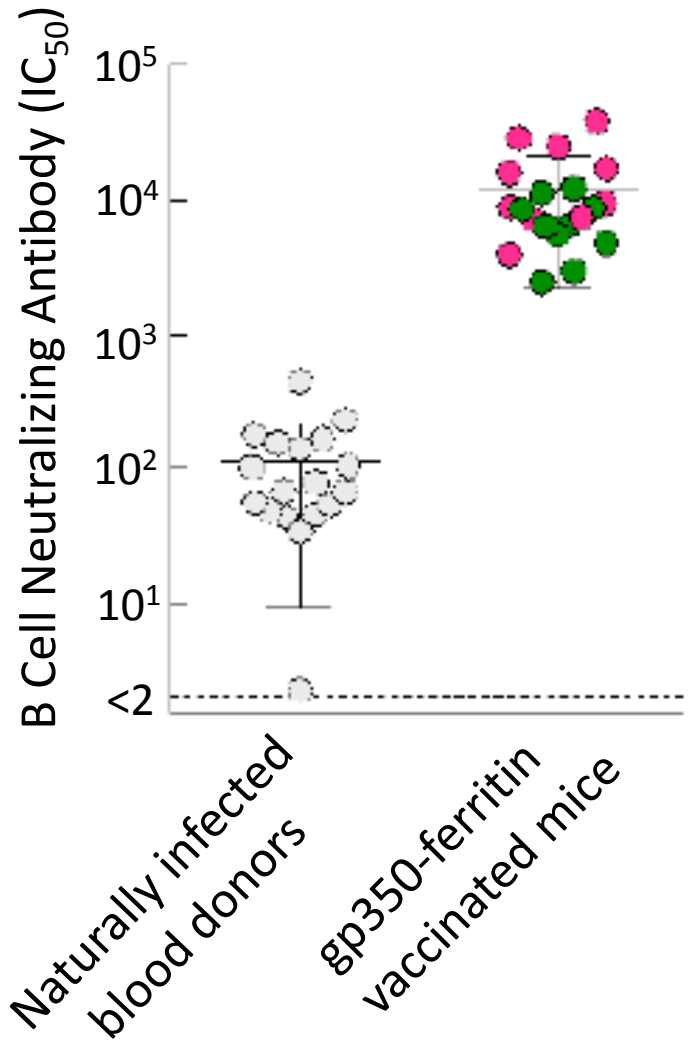


Ferritin-gp350 Protects Mice from Challenge with Vaccinia Virus Expressing gp350



	Mock	gp350 ectodomain	
gp350 ectodomain	ns		
D_{123}-ferritin	p = 0.0411	p = 0.0027	D_{123}-ferritin

Ferritin-gp350 Induces ~100-fold Higher B Cell Neutralizing Titers in Mice than Titers in Seropositive Humans



Should Other EBV Proteins Be Part of An EBV Vaccine?

Predominant Cells Infected by EBV

Cell Type	B Cell	Epithelial Cell
Natural infection	Resting B cell: primary infection latency	Oropharynx: primary infection shedding
Disease	Hodgkin lymphoma Burkitt lymphoma Post-transplant LPD	Nasopharyngeal carcinoma Gastric carcinoma Oral hairy leukoplakia
Entry		

Longnecker, Kieff, Cohen, Fields Virology 2013

Implications for EBV Vaccine

- EBV vaccine might induce antibodies to neutralize infection of both B cells and epithelial cells
  - **B cells**: prevent virus infection, latency, and lifelong infection
  - **Epithelial cells**: prevent viral infection and shedding

- To achieve this goal, EBV vaccine might be multivalent, including gp350, gH/gL ± gp42

Summary I

Soluble gp350:

- Protects cottontop tamarins from EBV-induced lymphoma
- Reduces EBV infection in the rhesus lymphocryptovirus model and reduces viral load in animals that become infected
- Reduces the rate of infectious mononucleosis in humans, but does not prevent infection

gp350 antibodies make up the predominant neutralizing antibody components in human sera to prevent EBV infection of B cells

Summary II

Antigen (3 doses)	Neutralization titer: Nanoparticle vs. soluble glycoprotein		Neutralization titer: Nanoparticle vs. seropositive human serum	
	B cell neutralization	Epithelial cell neutralization	B cell neutralization	Epithelial cell neutralization
Ferritin-gp350	100 ×	-	200 ×	-

Potential EBV Vaccine Trials

- Prevention of infectious mononucleosis in college-aged adults
- Prevention of post-transplant lymphoma in seronegative persons about to receive hematopoietic stem cell transplant
- Prevention of disease in seronegative boys with X-linked lymphoproliferative disease
- Prevention of Burkitt lymphoma in Africa
- Prevention of Hodgkin lymphoma, nasopharyngeal carcinoma, gastric carcinoma

Cohen, Clin Trans Immunol 2015

EBV gp350 Subunit Vaccine: Possible Outcomes in Humans

- Might not induce sterilizing immunity
- May limit level of replication and expansion of T cells during initial infection to reduce symptoms of IM
- May limit viral load setpoint after infection and reduce likelihood of PTLD
- May limit viral load setpoint after infection and reduce rate of EBV-associated malignancies (Hodgkin lymphoma, NPC, gastric carcinoma)

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